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C. F. A. PANTIN, Sc.D., F.R.S.

JOHN R. BAKER, D.Sc.

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The Giant Nerve-fibres in the Central Nervous System of Myxicola (Polychaeta, Sabellidae)

BY

J. A. C. NICOL

(*From the Department of Zoology and Comparative Anatomy, University Museum, Oxford*)

With fourteen Text-figures and three Plates

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I. INTRODUCTION

THE giant nerve-fibres which occur in the central nervous system of many Annelids are such large and conspicuous structures that they have aroused a considerable amount of interest and a large body of literature has grown up about them. During the latter part of the nineteenth century and the first years of this century, when the general morphology of the Annelida and the anatomy of the central nervous system of this phylum were being extensively investigated, there appeared frequent references to the occurrence of giant nerve-fibres in the Polychaeta. Most investigators were interested in classifying the species or in phylogenetic studies and merely recorded the presence or absence of giant fibres and giant nerve-cells. In many cases the size of the fibres was so great and they corresponded so little to the conventional picture of vertebrate peripheral nerve-fibres that their nature was in doubt for a long time and many hypotheses concerning their function were

postulated. For reviews of this controversial and nearly forgotten subject see Eisig, 1887; Friedländer, 1889; Lewis, 1898; Ashworth, 1909; and Stough, 1926. Many of these theories now seem fantastic, e.g. supporting functions and even excretory functions were suggested for the fibres. Such ideas were based entirely upon the appearance of the structures and in the absence of supporting experimental evidence each theory retained an element of probability. Friedländer (1889) suggested that the giant axons of Annelids were concerned with the quick end-to-end contraction ('startle-reaction') that earthworms and some Polychaetes exhibit when strongly stimulated. This theory received support from the experiments of Bovard (1918) who cut the nerve-cord of the earthworm and found a significant correspondence between the time of regeneration of the giant fibres and the return of quick contraction of the body, as contrasted with regeneration of the rest of the nerve-cord and the return of slower locomotory movements. Yolton (1923) was able to confirm this hypothesis by cutting the giant nerve-fibres without severing the rest of the nerve-cord; he found that the quick contractions failed to pass the site of injury. Subsequently, Eccles, Granit, and Young (1933), Rushton (1945, 1946), and Bullock (1945) have studied the action potentials of these fibres and have described their conduction properties.

Apart from the early detailed studies of the giant fibres of several different Polychaetes by Rohde (1887), Friedländer (1889), Gamble and Ashworth (1900), Ashworth (1909), and others, the majority of investigations have been confined to the Oligochaeta and recent physiological studies have been carried out exclusively on the earthworm. For this reason it was decided to examine the giant nerve-fibres of several Polychaetes with the aid of recently introduced histological methods. These giant fibres reach their greatest development in some sedentary Polychaetes (Spiomorpha and Sabellimorpha) and, since a number of early authors have commented on the extraordinarily large size of the giant axon of the Sabellid, *Myxicola infundibulum* Rénier, this species was selected for study.

II. Terminology

Knowledge of the nervous system of the Invertebrates has grown up with that of the Vertebrates and the resultant terminology is in part a borrowing from the Vertebrates and in part peculiar to the invertebrate forms; in either case it is frequently most inappropriate and misleading. It is proposed to use a consistent terminology in this paper for the features of the annelid central nervous system and the following terms have been adopted in preference to such ambiguous words as ventral chain, neurilemma, epineurium, and neurochords:

Supra-oesophageal ganglia, *oesophageal connectives*, *sub-oesophageal ganglion*, *nerve-cord*, *nerve-cord sheath*, and *peripheral nerves* refer to structures usually designated as such.

Ganglia of the nerve-cord. Swellings of the nerve-cord will be designated as ganglia only in those cases where there is definite evidence that they embody discrete accumulations of nerve-cells.

Giant nerve-fibres (or *axons*). Employment of these terms to designate the conspicuously large fibres of Annelids follows current English usage.

It is now necessary to define what is a giant nerve-fibre. First of all, it clearly refers to those nerve-fibres of any species which are disproportionately greater in size than the other nerve-fibres of the animal. They need not be great in absolute units, however. In the Enteropneusta, for example, they are 6μ or less in diameter (Bullock, 1944). But their size must be strikingly greater than that of the other fibres of the individual. Secondly, a functional distinction can be drawn, since in all Invertebrates in which this aspect has been investigated it has been shown that the giant fibres are concerned with quick escape or withdrawal mechanisms effected by widespread and synchronous or nearly synchronous muscular contractions (Yolton, 1923; Stough, 1930; Young, 1938, 1939; Ten Cate, 1938; Bullock, 1945; Rushton, 1946). It may be necessary to modify this criterion as more is learnt of the functions of these structures in various groups, but the existing evidence is compatible with this viewpoint. Hanström (1928) has suggested that there probably exist all gradations between ordinary nerve-fibres and giant nerve-fibres and Young (1936) found a continuous spectrum of fibre diameters in the nerve to the stellate ganglion of the cuttlefish. In this case, however, the giant axons 'were segregated by themselves in a corner of the nerve', while in the stellar nerves of the same animal the giant fibres were much larger than any others in the nerve. There is seldom any difficulty in determining whether any given species possesses a discrete system of nerve-fibres which can be termed giant axons. The following definition then would appear to satisfy existing knowledge: giant fibres are those fibres which have a much greater size than the other nerve-fibres of the animal; they form an essential nervous component of a system which effects a rapid, widespread, and synchronous response.

III. Historical Review

1. *Myxicola infundibulum* Rénier

Claparède (1861) was the first observer to record the presence of giant fibres in the nerve-cord of Annelids and in subsequent papers (1870, 1873) he gave a description of the nervous system and the giant fibres of *M. infundibulum*. According to this author the nerve-cord was double in the thorax, but one of the strands, after becoming enclosed within the sheath of the other, gradually tapered and disappeared; the other strand assumed a median position and continued posteriorly as the single nerve-cord. Ganglionic swellings were not pronounced and nerve-cells occurred throughout the length of the cord on the ventral and lateral surfaces. The giant fibre lay on the dorsal side of the nerve-cord (he regarded the giant fibre as being external to the nervous system) and had a diameter greater than that of the nerve-cord itself: 350μ in a specimen 5 mm. in diameter. The fibre was usually cylindrical throughout its length and was invested by a sheath composed of numerous layers of nucleated supporting tissue which was continuous with the external

covering of the nerve-cord. Two fibres arose in the supra-oesophageal ganglia and one passed down each oesophageal connective, there attaining a diameter of 55μ. On reaching the nerve-cord the two fibres united, with consequent fusion of their axoplasm. He examined fresh as well as preserved material and noted that the axon contained a clear, homogeneous substance.

McIntosh (1877) confirmed the fact that the nerve-cord was double in the anterior third of the animal and that each strand contained a giant fibre in its dorsal region. Posteriorly the two strands united and the resulting single cord contained a large, dorsally situated axon. According to Pruvot (1885), the central nervous system consisted of supra-oesophageal ganglia divided into pairs of superior, inferior, and posterior lobes, oesophageal connectives joining the nerve-cord in setiger II and a nerve-cord double throughout its length. Two pairs of peripheral nerves arose from the nerve-cord in each segment. The giant axon divided into two longitudinal fibres in the thorax; anastomoses connected the two fibres together in the commissures uniting the halves of the nerve-cord in setiger II.

Meyer (1887, plates; 1888, text) has described the general anatomy of the nervous system in some detail. He found that the supra-oesophageal ganglia were provided with abundant nerve-cells; that they gave rise to two pairs of coronal nerves; and that the posterior lobes were very well developed. This author also considered that the nerve-cord was double throughout its extent, with two pairs of ganglia and two commissures in each segment. This bipartite arrangement, however, was obscured in the abdomen by the very large median fibre which overlay the nerve-cords and it was only in the thorax, where the median fibre divided into two lateral halves, that the true bipartite condition was revealed. Of the two thoracic giant fibres, one ended blindly and the other proceeded posteriorly as the median giant fibre of this region. The oesophageal connectives arose from the nerve-cord in the middle of setiger II. His illustration shows a longitudinal division of the nerve-cord and the giant fibre in segments III and IV, with commissures between segments II and III, and between III and IV.

A similar description of the giant fibre was given by Cunningham (1888) who observed that there were two fibres in the anterior region of the body, one of which terminated without joining the other, while the other fibre continued caudad as the median giant fibre. He also confirmed Claparède's observation that the giant fibres in the oesophageal connectives became continuous with one another in the lower part of the supra-oesophageal ganglia.

Friedländer (1889) disagreed with Meyer's observations and stated that the two anterior giant fibres definitely fused with one another in the thoracic region. He showed, in addition, that the giant fibre contained an apparently homogeneous mass; that its sheath was relatively and absolutely much thinner than that of Lumbricus and Mastobranchus (Oligochaeta and Polychaeta, respectively, with strongly myelinated giant nerve-fibres); and that the sheath blackened only slightly during treatment with osmium tetroxide. He looked for, but could not find, the nerve-cells of the giant fibre.

Wawrzik's study (1892) of the giant fibre of this species was incidental to his investigation of the nature of fibrillae, a study which led him to believe that neurofibrils and connective tissue fibrils were continuous with one another and of the same nature. He described fibrils passing out of the giant fibre into its fibrous sheath. It is possible that he actually saw nervous processes connected with the giant fibre and interpreted them as connective tissue fibrils. His photomicrograph of a transverse section shows the giant fibre badly distorted and shrunken from its sheath, except at a ventro-lateral corner. Now it is in this latter region that large peripheral branches arise from the giant fibre. He apparently observed such a branch and interpreted it in support of his hypothesis.

The erroneous descriptions of Claparède and Meyer, in placing the giant fibre outside the nerve-cord, can be attributed to uncertainty concerning the functional significance of this structure. It is difficult, however, to understand the reasons which led Meyer and Pruvot to conclude that the nerve-cord of this species was double throughout its length. They were probably influenced by the prevalent concept that the nerve-cords of all Annelids consisted of a ganglionic chain and they interpreted the longitudinal fibrous tracts of the cord as separate halves of the nerve-cord. It is certain, however, that double ganglia, transverse commissures, and a ladder-like structure simply do not exist as constant metameric characteristics in this species. All authors agreed, however, that the nerve-cord bifurcated anteriorly. Slight differences exist in the pictures given by Pruvot and Meyer, but both investigators have shown a double nerve-cord anterior to setiger IV, with two transverse commissures somewhere in setigers II and III. Friedländer (1889) has shown that the two thoracic giant axons actually fused together, contrary to the descriptions of Meyer and Cunningham. Although there is a certain amount of variation in the course of this fibre, and it is possible that the rare individual might actually show discontinuity of fibres in this region, a more likely explanation is that the latter two authors had based their descriptions on an incomplete series of sections, or on poorly fixed material. Another factor which may have led them into error is the fact that occasionally one of the longitudinal divisions of the nerve-cord in the anterior thorax is much smaller than the other and lacks a giant fibre. Isolated sections from such a region could provide a basis for the interpretations of these authors.

The following description of the central nervous system of *M. infundibulum*, therefore, can be compiled from the several accounts:

1. The supra-oesophageal ganglia lie in the prostomium and consist of a pair of superior and a pair of inferior ganglia from which arise prostomial and coronary nerves, and a pair of posterior lobes.
2. Oesophageal connectives join the nerve-cord in setiger II.
3. The nerve-cord is single throughout most of its length but is double in part of the first four thoracic segments where two commissures unite the two halves of the cord.

4. A giant fibre of exceptionally large size extends throughout the central nervous system. It arises in the supra-oesophageal ganglia as two small fibres, one of which extends down each oesophageal connective. The two fibres fuse with one another in the sub-oesophageal ganglion and the resultant fibre extends to the posterior end, dividing where the cord divides, and fusing where the cord is single. It possesses a distinct sheath.

In a short preliminary note to the present paper, Nicol and Young (1946) have confirmed the description given above of the giant fibre of *Myxicola infundibulum*, and have shown in addition that the sheath of the fibre was negative towards osmium tetroxide and did not possess a pronounced myelin sheath. They found that the giant fibre was a syncytial structure, connected with small nerve-cells throughout its length, and they substantiated this fact by the observation that the giant fibre did not degenerate after section during periods up to 16 days following injury. They observed, moreover, that the fibre gave off branches to the peripheral nerves along its entire course.

Several authors have stated that this species gives a very vigorous and quick contraction when mechanically stimulated (Montagu, 1808; Friedländer, 1889). Claparède (1870) found that during this contraction the animal shortened its length by half. Since it has been proved definitely that the giant fibres of earthworms are concerned with the transmission of impulses concerned with the quick end-to-end contractions of the body, there is little doubt that the giant axon of *M. infundibulum* also forms part of the nervous pathway for the vigorous contractions of this species.

2. *M. aesthetica* Claparède

M. aesthetica is a small replica of the larger species, *M. infundibulum*. The internal anatomy of this species is largely unknown. De St. Joseph (1894) has given several details concerning the blood vascular system, but the nervous system has never been described. Okada (1932, 1934), in an extensive and dexterous investigation, has shown that this animal possesses remarkable powers of regeneration. Any body segment could give rise to a new head anteriorly and a new tail posteriorly, and several heads could be induced to grow on the same animal by cutting the nerve-cord. He considered that the nerve-cord was necessary for regeneration to proceed to completion, but he did not concern himself with the structure and growth of the internal organs.

It is proposed in this paper to give a description of the main features of the central nervous system of *Myxicola infundibulum* and *M. aesthetica*, in so far as they are related to the arrangement of the giant nerve-fibres of these species. The structure of the giant axons has been investigated with the following points in view, in order to establish an anatomical background for the study of behaviour and nervous function of the Sabellidae: (1) size and course of the giant axons; (2) sheaths; (3) nerve-cell bodies; (4) syncytial or

unicellular nature; (5) branches or efferent connexions. It is hoped to present further data concerning the functioning of these structures in a subsequent communication.

IV. Material and Methods

The specimens of Myxicola used in this investigation were obtained from the Plymouth Laboratory of the Marine Biological Association. *Myxicola infundibulum* is a tubicolous form dwelling in sand–clay flats near low spring-tide mark. Montagu (1808), who first discovered this species on the Saltstone near Salcombe, Devon, has given a good description of its occurrence there. It is distributed fairly abundantly over a short stretch of the shore, and the burrows of the animals may be readily detected by the slight protrusion of the blackish gelatinous tube which projects above the surface. The animal lives in a long and very voluminous mucous tube which fills its burrow. Mature specimens are about 10 cm. to 13 cm. long when normally extended, and about 6 mm. wide. The mean number of segments lies near 130. There are 8 setigerous segments in the thorax which show the usual Sabellid characteristics. It lives well in the laboratory for several months when kept in running sea-water. A group of animals usually forms a thick mucous mass which tends to float at the surface, but this difficulty can be overcome, however, by supplying the animal with sand in which to burrow.

M. aesthetica resembles, in miniature, the larger species, *M. infundibulum*. Specimens were obtained in dredgings from Asia shoal, particularly in association with Ascidiella. It is tubicolous, secreting a hyaline, jelly-like tube. Larger specimens are about 15 mm. long and 1 mm. wide and have up to 50 segments. The thorax consists of 3 setigerous segments. This species is very hardy and will live for long periods in finger-bowls containing sea-water.

The nervous elements of the annelid nervous system stain poorly with routine stains and require special techniques in order to render the individual neurones distinguishable. Since methods of silver impregnation are usually necessary, the fixatives which can be employed are limited by this factor as well as by the necessity of obtaining adequate preservation of histological and cytological detail. The several techniques which were tried are discussed as follows.

1. *Fixatives*

The necessity of obtaining good fixation is well emphasized by the errors which have crept into the work of several earlier investigators who have drawn conclusions regarding normal structure from patent artifacts. Rohde (1887), for example, has described nerve-fibres traversing large spaces which he found between the giant axon and its sheath in certain Polychaetes, spaces obviously due to shrinkage of the axoplasm during fixation and subsequent treatment. A number of investigators have stated that the fixative should be isotonic, as regards its neutral salt content, with the tissue studied in order to obtain optimal preservation (Carleton, 1922; Young, 1935; Cole, 1946; and others). With the exception of special cases where other factors were involved, all

fixative solutions employed in this study were made isotonic with sea-water, either by making up the solution in sea-water itself or by the addition of a suitable quantity of some indifferent salt.

A series of commonly used fixatives was tried out on *M. infundibulum* in an attempt to discover one which would give both reasonably good fixation and permit subsequent treatment with silver solutions. The two solutions which were found to be most satisfactory, in consequence, were Bouin's fluid and picro-formol, both of which were made up in sea-water. Picro-formol consisted of a saturated solution of picric acid in sea-water, 90 parts, and commercial formalin, 10 parts. These two reagents gave good fixation of the nervous system and the giant fibre, and it was possible to impregnate the neurones with silver after removal of the picric acid in 70 per cent. alcohol. A series of specimens of several species of Sabellids was fixed in chilled Bouin's fluid as recommended by Bodian (1937*a*), but no significantly better results were obtained by this treatment than by using the fixative at room temperature.

Simple solutions of formalin and picric acid caused considerable distortion of the giant fibre of *M. infundibulum* when paraffin embedding was employed. Young (1939) found formol and picro-formol solutions successful for preserving Cephalopod giant fibres, and Holmes (1942) obtained good results on prawn fibres with picric acid. Since neither formol nor picric acid alone permitted satisfactory silver impregnation in Myxicola, however, they were discarded. Flemming (without acetic) was also found to cause considerable shrinkage of the axon.

Helly's and Zenker's fluids, and post-chroming after formol-fixation, were also discovered to be good fixatives for the central nervous system. Mallory's triple and Heidenhain-azan stains gave satisfactory results after fixation in these media. These fixatives, however, greatly inhibited effective silver impregnation, properties already well known for the vertebrate central nervous system (Foley *et al.*, 1936; Bodian, 1937, 1937*a*).

Osmium tetroxide, in solutions of strength 0·2 to 1 per cent., was used for detecting the presence of myelin sheaths. Preservation was poor, however, and better results were obtained by employing the mixture of osmium tetroxide and picric acid recommended by Holmes (1942).

2. *Embedding media*

In an effort to eliminate some of the distortion caused to such a large, homogeneous mass of protoplasm as the giant nerve-fibres of the Polychaeta by paraffin embedding, some of the material was cut in Péterfi's celloidin-paraffin and in celloidin. Double embedding in methyl benzoate-celloidin gave very good results and recourse was had to celloidin sections for checking critical points only.

3. *Staining*

Previous workers who have investigated the annelid nervous system have found that the nerve-cells and their processes are difficult to stain and it is

only in exceptional circumstances, e.g. in the case of the giant nerve-cells of Lumbriconereids (Fedorow, 1928) where peculiarities of size facilitate identification, that giant axon neurones can be distinguished after routine methods. Earlier workers tried to reveal the nervous elements in the annelid nervous system by metallic impregnation, either with OsO_4 (Friedländer, 1888, 1894), or by the chrome-silver of Golgi (Nansen, 1887; Retzius, 1892), or silver methods of Cajal (Ramón y Cajal, 1904; Boule, 1908, 1909; Kowalski, 1909). *Intra vitam* methylene blue has been used with variable results. Retzius (1891) and Hamaker (1898) employed it successfully on Nereis and Krawany (1905) on the earthworm, but in general the method gave incomplete or negative results on Polychaetes (Retzius, loc. cit.; Evenkamp, 1931). More recently, methods of impregnating sections with silver have given good results among Invertebrates (Bodian, 1937; Bullock, 1944, 1945, 1945*a*; Holmes, 1942).

Two silver methods have been used extensively in this study, viz. impregnation of sections by activated protargol, and by Holmes's buffered silver nitrate-pyridine solutions. As might be expected, these gave different results in different species of Polychaetes and required to be modified in each case for the optimal effect. Impregnation was most satisfactory after fixation in picro-formol or Bouin's solutions, but a small degree of silver impregnation was also obtained in Helly-fixed material after subjecting the sections to 5 per cent. sodium bisulphite for 24 hours to remove the chrome salts, as recommended by Bodian (1937*a*). Counter-staining with Heidenhain-azan in this case gave a more complete picture. Nuclei, processes, giant fibres, and cytoplasm were all darkened with silver, and usually in that order of intensity, the smaller processes more intensely than the giant fibres.

Activated protargol was used in the manner recommended by Bodian (1936, 1937), employing Bayer's protargol (silver proteinate). The writer is indebted to Dr. William Holmes for assistance in using his method of silver impregnation which has since been published (Holmes, 1947). The basic method employed is as follows:

1. Place sections in 20 per cent. $AgNO_3$, in the dark, for 1½ to 2 hours.
2. Wash, 10 min., in aqua dest., 3 changes.
3. Incubate, about 24 hrs., at 37° C., in a medium containing:

Boric acid, 12·4 gm. per l.	55 c.cm.
Sodium borate, 19 gm. per l.	45 c.cm.
Pyridine, 20 per cent. aqueous solution	5 c.cm.
Silver nitrate, 1 per cent.	2·5 c.cm.
Aqua dest.	392·5 c.cm.

   Strength of $AgNO_3$, 1/20,000
   pH of buffer mixture, 8·4
4. Wash, aqua dest., 3 min.
5. Reduce, 3 min., in

Hydroquinone	1 gm.
Sodium sulphite, 20 per cent.	100 c.cm.

6. Wash, tap-water, 3 min.
7. Rinse, aqua dest.
8. Tone, gold chloride, 0·2 per cent., 3 min.
9. Rinse, aqua dest.

10. Place in oxalic acid, 2 per cent., until sections become purple.
11. Two rinses, in aqua dest.
12. Fix in sodium thiosulphate, 5 per cent., 5 min.
13. Wash, tap-water, 10 min.

The sections are then ready for counterstaining or mounting.

The following paragraphs refer to the effects of these reagents on the two species.

Myxicola infundibulum. Reasonably good impregnation was obtained by both protargol and the basic buffered solutions and the results were similar in both cases. A deeper degree of impregnation resulted from increasing the silver strength of the buffer solution to 1/10,000.

M. aesthetica. The giant fibres and the nerve-cell nuclei were darkened by the basic buffered solution, but the cytoplasm of the nerve-cells and their processes were refractory to impregnation, even when the other tissues absorbed the silver heavily. Counterstaining with Heidenhain-azan revealed additional features. In the latter case it was found advisable to carry through a slide of *M. infundibulum* simultaneously in order to control the staining, since sections of *M. aesthetica* are very small and it is difficult to observe the differentiation of the stain under low-power objectives.

4. *Special methods*

Several methods were employed to investigate the problem of myelination. Besides fixation in osmium tetroxide, a method of greater morphological value than histochemical accuracy (Owens and Bensley, 1929; Lison, 1936; Dempsey and Wislocki, 1946; Baker, 1944), material was treated with Sudan IV and Sudan black and carried through Baker's test for lipines. Baker has described his method fully (1946) and his directions were followed carefully. In the case of sections to be treated with Sudan dyes, small pieces of the animal were fixed in Baker's formol-calcium and stored in formol-calcium-cadmium (Baker, 1944). After embedding in gelatine, the tissue was cut on the freezing microtome, mounted on slides, and treated with saturated solutions of Sudan black and Sudan IV in the manner recommended by Pantin (1946).

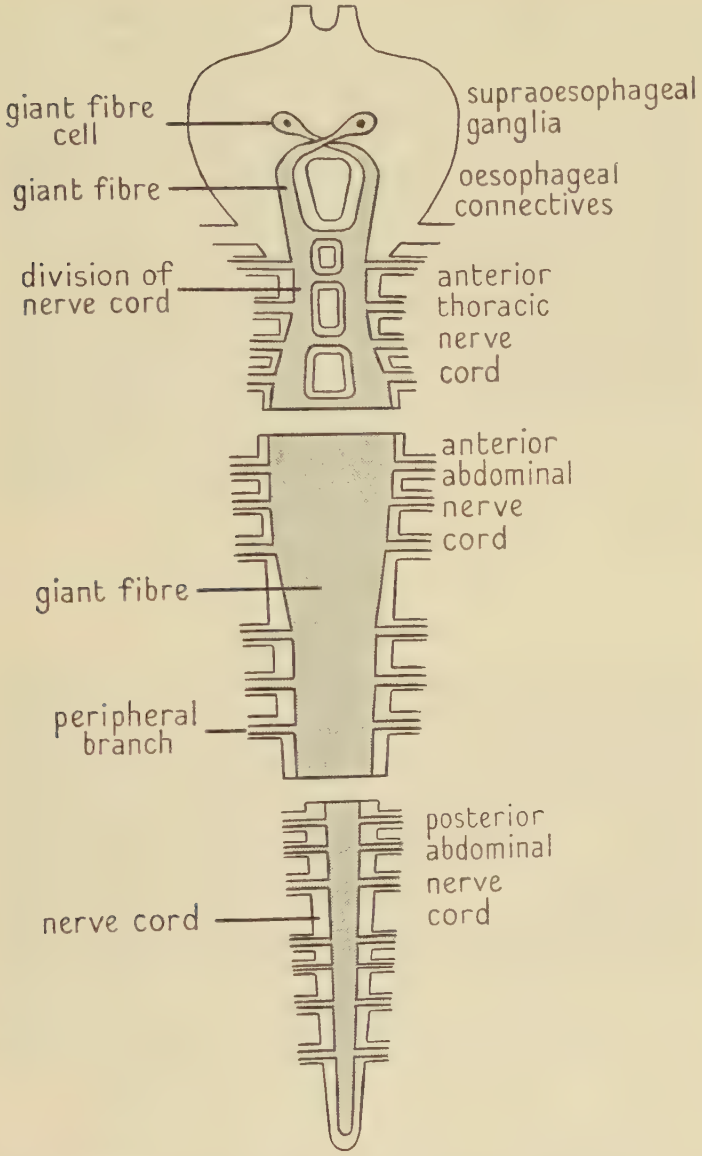
V. Observations

1. *Myxicola infundibulum* Rénier

(*a*) *Description of the central nervous system*

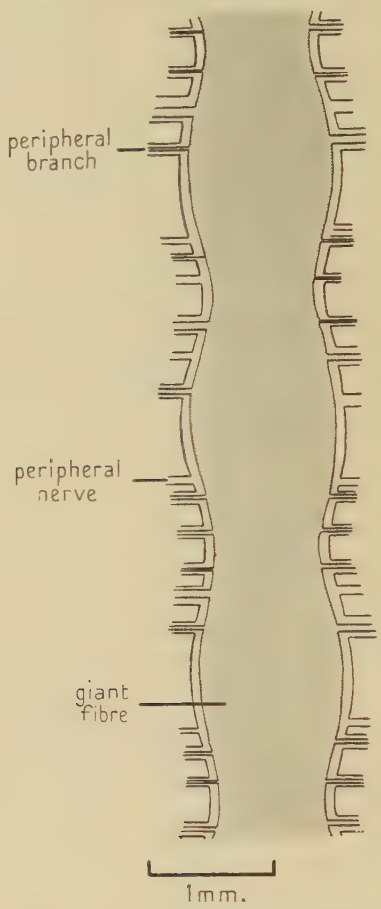
The central nervous system conforms to the typical annelid pattern and consists of supra-oesophageal ganglia, oesophageal connectives, sub-oesophageal ganglion, and a nerve-cord lying in the ventral body-wall (Text-fig. 1A).

The supra-oesophageal ganglia lie above the gut in the prostomium, and extend caudally into setiger I. They may be divided into a pair of inferior ganglia and a pair of superior ganglia (Text-figs. 2A and 2B). These are connected with one another by a median bridge of nervous tissue. The ventral, posterior regions of the inferior ganglia pass gradually into the oesophageal



TEXT-FIG. 1A. Diagram of the central nervous system and giant fibre of *Myxicola infundibulum* as seen from above. The nerve-cord is shown divided in three places in the thoracic region.

connectives which curve obliquely ventrally and posteriorly, and unite with the sub-oesophageal ganglion in setiger II (Text-figs. 3A and 3B). The general configuration of the supra-oesophageal ganglia is that of a short, broad mass, whose superior ganglia form a relatively high saddle embracing the excretory

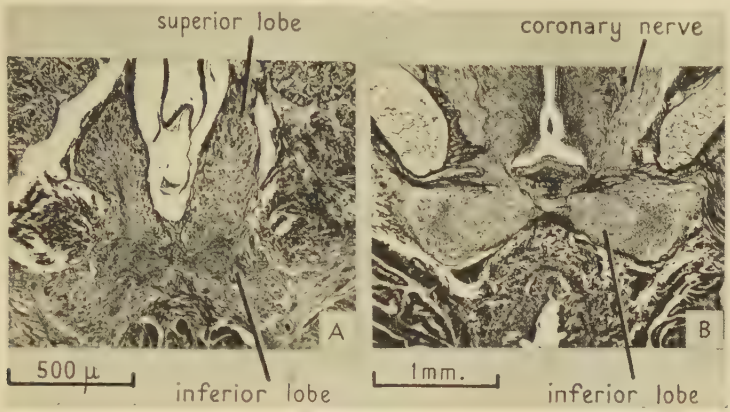


TEXT-FIG. 1B. Reconstruction of the nerve-cord and giant fibre in three anterior abdominal segments. Dorsal view.

duct of the large anterior nephridia. In a mature animal they are about 1·5 mm. broad, 0·5 mm. long, and 1 mm. high.

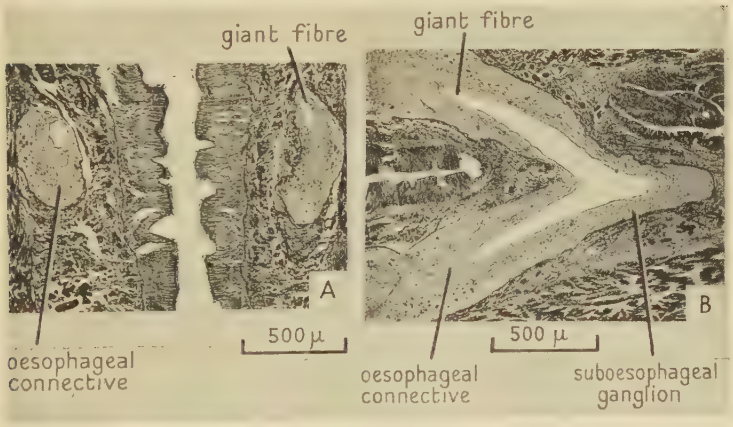
The nerve-cord for purposes of description may be divided into two regions: the thoracic nerve-cord and the abdominal nerve-cord. In the thorax the cord divides into two at several levels; in the abdomen it is usually single. The sub-oesophageal ganglion, lying in setiger II, is a relatively broad mass larger than the rest of the nerve-cord. Behind this ganglion, in setigers II to IV, inclusive, the nerve-cord divides into two portions on three or four occasions. These divisions are fairly constant, but do not correspond exactly

n position, extent, and symmetry in different individuals (Text-figs. 1A and 1A). The following figures are typical and have been worked out in detail rom serial sections of a mature animal. The first double region is about



Text-fig. 2A. Photograph of a transverse section through the supra-oesophageal ganglia of *M. infundibulum*. Picro-formol, cedarwood oil, paraffin, Holmes's silver, safranin.

Text-fig. 2B. A horizontal longitudinal section through the same structure. Helly's, cedarwood oil, paraffin, Holmes's silver, Heidenhain-azan.



Text-fig. 3A. Photograph of a horizontal longitudinal section through the oesophageal connectives of *M. infundibulum*. Helly's, cedarwood oil, paraffin, Holmes's silver, Heidenhain-azan.

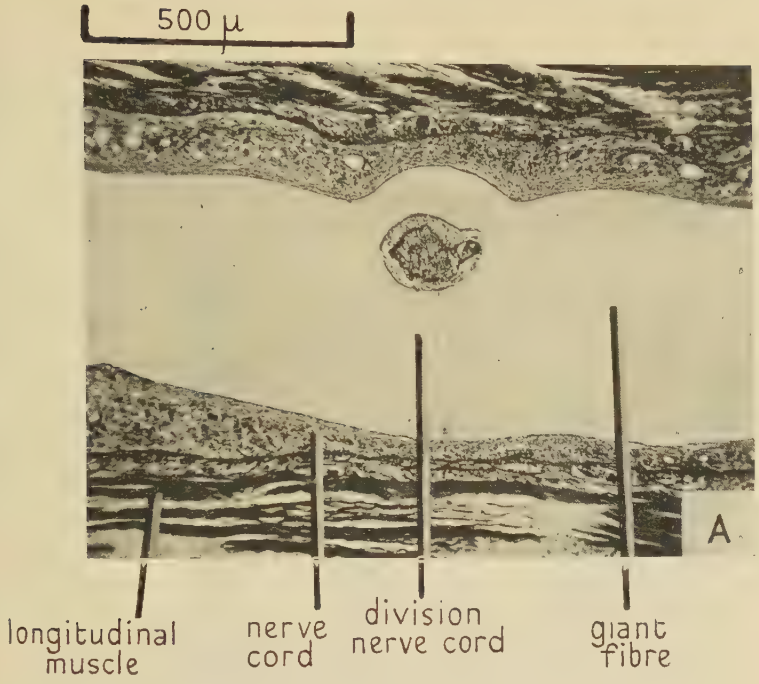
Text-fig. 3B. The same specimen. A section more ventrally through the sub-oesophageal ganglion.

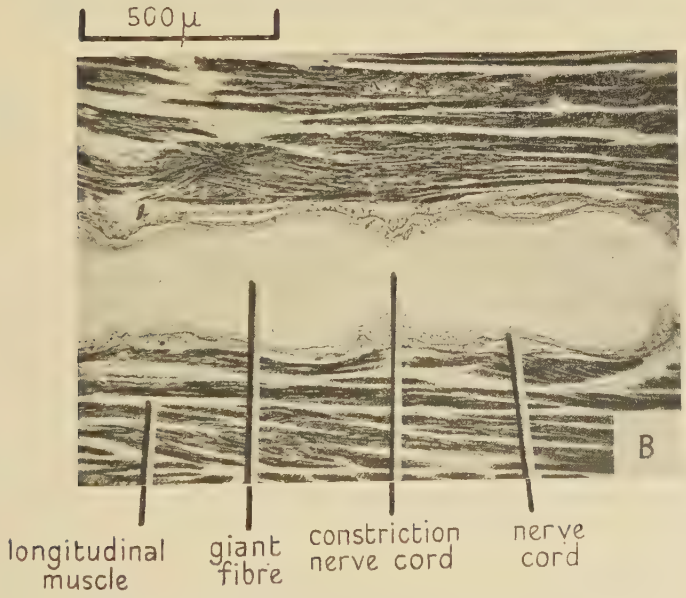
50μ long and lies immediately behind the sub-oesophageal ganglion. The following two divisions are longer, about 100μ to 150μ in extent, and occur one per segment up to and including setiger IV. These separations of the cord show considerable variation in that they may be central and thus divide the cord into two equal portions; or they may lie laterally, giving rise to a

small lateral strand of nerve-cord which appears like an appendage of the main strand. Behind setiger IV, the cord remains single with the exception that irregularly, here and there, over stretches of less than 1 mm., a small, ventro-lateral strand of the nerve-cord may become cut off from the main body of the nerve-cord, only to rejoin it again.

This irregularity in pattern explains some of the discrepancies in the descriptions of Claparède, Pruvot, Meyer, and Friedländer, discussed previously. It is obvious that the bifurcations of the nerve-cord are not constant in number or in position. The features that they have in common are these: they are more extensive in the anterior thoracic segments than farther posteriorly; and they cause the nerve-cord, in the first four thoracic segments, to assume a bipartite condition with transverse commissures in each segment. The most satisfactory explanation of this condition would be given by knowledge of the development of the nerve-cord, but unfortunately the development of Myxicola is unknown. It may be suggested, tentatively, that the nerve-cord arises, as in other Polychaetes, from bilateral Anlagen in each segment, and that the adult condition is the result of a gradient in the degree of fusion of these embryonic neural masses, a fusion which proceeds farther in the abdomen than in the thorax. It is interesting to note, in this connexion, that the nerve-cord and its contained giant fibre frequently appear divided in the posterior few segments where it would be expected that increment in length occurs. At least in the related species, *Branchiomma vesiculosum* Montagu, Wilson (1936) has stated that new segments are laid down immediately in front of the pygidium after metamorphosis.

Meyer (1888) and Pruvot (1885) have described two pairs of nerves per segment. Careful reconstructions of serial sections show, however, that the true condition is more complicated. The number of peripheral nerves in each segment is not constant. There are actually five to seven nerves arising from each side of the nerve-cord in each metamere and these are of variable size, some containing one or a few fibres, others a large number. The mean number is six pairs and they are grouped into two fields. There is an anterior set of about three nerves on each side, occurring from the level of the intersegmental septum into the anterior third of the segment. Separated from this set by a considerable interval is a posterior set of another three nerves lying in the posterior third of the segment, in front of the posterior septum of that metamere (Text-fig. 1B). This may be compared with the condition which obtains in the Maldanids studied by Lewis (1898), but in these forms the arrangement was even more irregular and no pattern could be discerned. The peripheral nerves arise from the ventro-lateral border of the nerve-cord and proceed dorsally, between the circular and longitudinal muscle layers, towards the dorso-medial surface of the animal (Text-fig. 11; Pls. 1, 2 and 3, figs. 1, 12, 13, and 18). Besides these nerves, groups of fibres and single fibres leave the nerve-cord either ventrally to enter the sub-epidermal tissue, or laterally to innervate the longitudinal muscles lying immediately lateral





Text-fig. 4. Horizontal longitudinal sections through the thoracic nerve-cord of *M. in-undibulum*. Helly's, cedarwood oil, paraffin, Holmes's silver, Heidenhain-azan. Text-fig. 4A s from a region lying slightly more anterior to that shown in 4B.

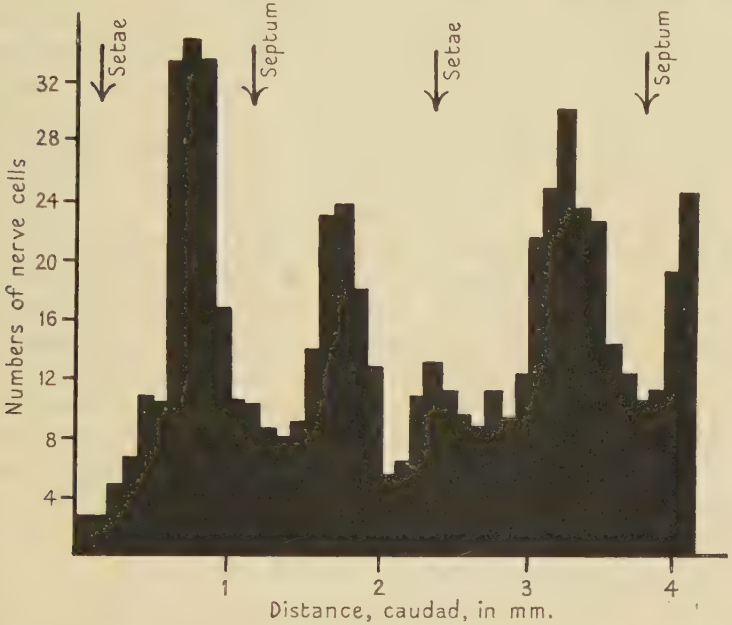
to the nerve-cord, or the circular muscles occurring in the intersegmental septa.

Several features of the nerve-cord of this animal can be observed in the living state by careful dissection. Because the animal contracts so violently it is necessary to narcotize it as a preliminary: immersion in 5 per cent. ethyl alcohol in sea-water for 15 minutes is effective. It is then slit open along the dorsal mid-line, which can be readily identified from the position of the ventral faecal groove, and the intersegmental septa are transected on either side of the gut. The animal is pinned out in a dissection dish and the gut stripped off with bent forceps under a binocular microscope. The nerve-cord is revealed by the position of the ventral blood-vessel which clings to its superior margin; it lies flush with the peritoneal surface of the longitudinal musculature. Two facts are then apparent. First of all, the nerve-cord is swollen in each segment, between the levels of the anterior and posterior septa, forming a beaded strand; secondly, it contains a large, clear, hyaline tube, the giant fibre, which is similarly expanded in each segment, forming a series of segmental swellings. In an attempt to determine the nature of these segmental swellings, the nerve-cords of several specimens have been reconstructed by plotting the horizontal plane of greatest diameter in transverse serial sections. Text-fig. 1B represents the result of such a reconstruction in the region of three anterior abdominal segments of a mature animal killed and fixed in a semi-contracted condition. The nerve-cord here shows constrictions at septal levels and swells out in the middle of each segment, the diameters alternating from about 1·3 mm. to 1 mm. It will be noticed, in addition, that the giant fibre shows the same alternations in diameter and follows closely the variations in cord volume (Text-fig. 4B).

Before considering, however, whether such swellings can be designated correctly as ganglia, it is necessary to consider their nerve-cell content. The usual significance of the term ganglion is that of an accumulation or aggregation of nerve-cells (Maximov and Bloom, 1944, p. 216). Nerve-cells are abundant in the supra-oesophageal ganglia (cf. Meyer, 1888; Johansson, 1927) and in the sub-oesophageal ganglion, and occur throughout the length of the nerve-cord. An enumeration of the total number of nerve-cells in several segments was made by counting all the nerve-cell nucleoli in a series of transverse serial sections, and the results are shown in Text-fig. 5. The nerve-cells reach their greatest density in the posterior region of the nerve-cord of each segment, in front of the intersegmental constriction, decrease in numbers at the septal level, and show a secondary and lesser accumulation in the anterior portion of each segment at the level of the setae. Accumulations of nerve-cells are thus apparent in the anterior and posterior portions of each segment, and these correspond, respectively, with the anterior and posterior grouping of the peripheral nerves. On the other hand, they are not directly correlated with the segmental swellings of the nerve-cord, most of which can be related to the bulging of the giant axon in the centre of each segment. This pattern possibly corresponds to the arrangement which occurs

other Sabellids (e.g. Sabella, Thomas, 1940; Laonome, Evenkamp, 1931) here there are a pair of anterior and a pair of posterior ganglia per segment, but it is so much less clearly marked that it does not warrant the pecial designation of ganglia.

The nerve-cord lies in a ventral cleft within the longitudinal muscle layer nd it is separated from the latter by a narrow coelomic space which descends



TEXT-FIG. 5. Histogram showing the distribution of nerve-cells at successive levels in the rve-cord. The middle of each segment (setae) and the intersegmental level (septum) are arked by arrows.

most to the level of the circular muscles (Text-fig. 11; Pl. 1, figs. 1, 5; . 3, fig. 13), except at intersegmental levels where the septum bridges the ap between the nerve-cord and the body-wall. It is attached above by a entral, longitudinal mesentery throughout its length, which contains chloragen cells and the ventral blood-vessel. The nerve-cord is surrounded xternally by a sheath consisting of three components. Externally lies a layer flattened epithelial cells. Beneath this peritoneum there is a thin sheet of onnective tissue enclosing a layer of circularly and longitudinally arranged uscle-fibres and small blood-vessels. This layer expands below, where it ntains a relatively large amount of circular muscle-fibres which are conuous with those of the body-wall. It fuses with the septa at intersegmental vels and the enclosed muscle-fibres extend out as a broad sheet within the pta. Within this layer is the tunica propria of the nerve-cord. This consts of a compact band of connective tissue completely investing the whole rd with the exception of the places where the peripheral nerves emerge. It

forms a distinct sheath, sharply marked off from the other layers about the nerve-cord. It is composed rather of a sheet of tissue than of separate fibres and is probably collagenous since it shows a marked affinity for the aniline blue and acid fuchsin of Mallory's triple and van Gieson's connective tissue stains, respectively. Sections treated with Taenzer-Unna's orceïn for elastin failed to show any elastic tissue fibres.

The nerve-cord contains four separable components: the nerve-cells and their processes; the neuropile; the giant fibre and its sheath and branches; the supporting elements. In a cross-section it is seen that the giant fibre occupies a relatively large volume of the nerve-cord and lies dorsally to the nerve-cells and neuropile (Text-fig. 11; Pl. 1, figs. 1 and 5; Pl. 3, fig. 13). Laterally and superiorly it is situated close to the sheath of the nerve-cord from which it is separated by a layer of fine fibres and its own fibrous and cellular sheath. The nerve-cells lie ventrally and laterally in the cord, decrease in numbers dorsally, and in the upper half of the cord they are very sparse, only one or two occurring here and there in the narrow space between the giant fibre and the sheath of the nerve-cord (Pl. 1, fig. 1). The neuropile forms the central mass of the ventral half of the cord. It contains longitudinally and transversely running nerve-fibres, among which may be distinguished fibres which (1) extend from one segment to the next; (2) cross the nerve-cord; (3) enter into the peripheral nerves; (4) connect with nerve-cells in the cord; (5) join the giant nerve-fibre. The supporting tissue is composed of very slender fibrils and small cells which lie among the nerve-cells and about the giant nerve-fibre.

(*b*) *Arrangement of the giant axon*

The giant nerve-fibre forms the most conspicuous and the predominating feature of the central nervous system of this species. It arises in the supra-oesophageal ganglia, extends down the oesophageal connectives into the

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PLATE 1. *Myxicola infundibulum*

All figs. on this plate are from sections of material cleared in cedarwood oil and embedded in paraffin wax, unless otherwise stated.

Fig. 1. T.S. of the nerve-cord showing the giant axon and the origin of the peripheral nerves from the cord. Helly's, Holmes's silver, Heidenhain-azan.

Fig. 2. Horizontal longitudinal section through the supra-oesophageal ganglia showing the decussation of the two giant axons in this region. Helly's, Holmes's silver, Heidenhain-azan.

Fig. 3. Horizontal longitudinal section of the nerve-cord. Continuity of axoplasm of the giant fibre and a peripheral branch is shown clearly. Picro-formol, Péterfi's celloidin-paraffin, Holmes's silver.

Fig. 4. Sagittal section showing two successive branches of the giant axon. The fibre from the right-hand branch extends longitudinally through the nerve-cord, and joins the left-hand branch in the next section. Picro-formol, Péterfi's celloidin-paraffin, Holmes's silver.

Fig. 5. T.S. of the giant fibre showing striations in the axoplasm. Flemming's (without acetic), iron haematoxylin and orange G.

Fig. 6. Composite picture built up by superposition of photomicrographs of three serial and consecutive sections. The giant fibre branch on the left subdivides into three fibres, and also sends a fibre diagonally across the nerve-cord towards the opposite side. Helly's, Holmes's silver, Heidenhain-azan.

erve-cord, and runs through the entire length of the latter structure (Text-g. 1A).

Within the supra-oesophageal ganglia the giant fibres lie in the ventro-osterior border of the inferior lobes (Pl. 1, fig. 2, and Pl. 3, fig. 14). They re very slender, about 15μ in diameter, and extend transversely through hese ganglia across the mid-line. Near the median plane they are connected ith two relatively large cells which occur ventrally and anteriorly to them, nd which also have their axes of greatest length arranged transversely (Pl. 3, g. 15). On descending into the oesophageal connectives each fibre gives off number of branches which proceed dorsally and anteriorly within the europile of the supra-oesophageal ganglia and oesophageal connectives. The bres lie on the median face of the connectives and, in the sub-oesophageal anglion, the two fibres fuse together in the mid-line (Text-figs. 3A and 3B).

A short distance in front of the point where the oesophageal connectives rise from the anterior end of the nerve-cord they are connected together y a narrow transverse bridge. This commissure contains at least one fibre hich connects together the giant fibres of each side. It is difficult to resolve he true picture here because of the multitude of fibres present, but in hori-ontal longitudinal sections it can be seen definitely that a slender branch rom the giant fibre in each oesophageal connective curves ventrally and osteriorly, passes into this commissure, and meets a corresponding fibre from he other side in this latter structure.

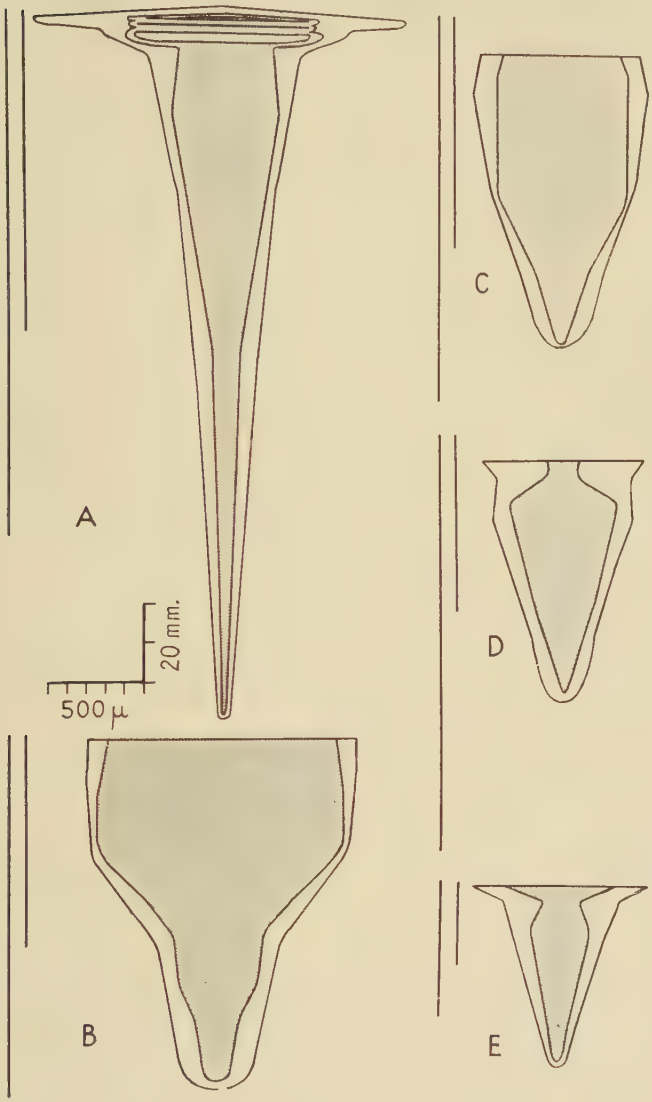
In thoracic segments II to IV the giant fibre usually branches where the erve-cord divides into two and a fibre runs through each half of the nerve-ord (Text-figs. 1A and 4A). The two fibres so formed join together where he two cords fuse or, to use the conventional nomenclature, they are con-ected together by commissures. But when one of the strands of the nerve-ord is smaller than the other, as frequently happens, the giant fibre may xtend through one strand of the cord only and thus remain single even when he cord is divided. The pattern of the giant fibres in the thorax thus shows considerable amount of individual variation and it is impossible to present rigid description that is applicable to every case. At those places where the iant fibres bifurcate and fuse again it is certain that there is continuity of xoplasm, and that the several portions are united to one another without the nterposition of intervening septa or separating membranes (Text-fig. 4A). his is clearly seen in all preparations, fixed and stained by different methods, nd cut in three planes at right angles to one another. Posterior to segment IV he giant fibre remains single and median in position throughout its length Text-fig. 4B).

The following figures, obtained from a mature specimen, are representative f the relative diameters of the giant fibre in different regions of the body. he giant fibres are small in size in the dorsal half of the oesophageal con-ectives (about 60μ), increase in diameter as they pass ventrally (about 70μ), nd, just before fusing in the sub-oesophageal ganglion, each fibre attains a iameter of about 100μ. Immediately after the fibres fuse the diameter is

200μ. The fibre continues to expand in the thorax, having a width of 300μ where single and a combined diameter of about the same where bipartite. It reaches its greatest width in the posterior thorax (500μ), then gradually decreases to 100μ in the middle of the abdomen and very gradually tapers off posteriorly.

The figures given above are not constant, for there is a considerable amount of individual variation (in one case a diameter of 1·7 mm. was measured), and the measurements obtained also depend on whether the animal was killed in a contracted or extended state. In order to determine the variation in size of the giant axon in animals of different lengths and the alteration in diameter that occurs during contraction and extension, a number of animals of different sizes were killed in extended and contracted conditions. Sample sections were cut at regular intervals along the length of these specimens and the maximal horizontal diameters of nerve-cord and giant axon were measured and plotted against length. The results are shown in Text-fig. 6. To afford an indication of the size of the animal its extended and contracted length were determined before death and are marked on the left-hand side of each figure. Text-fig. 6A represents the nerve-cord and giant axon of the largest animal, abnormally extended under anaesthesia. The giant axon has a maximal diameter of 560μ in the thorax and decreases to 140μ in the mid-abdomen. B and C were obtained from smaller specimens of about equal size. In these animals the giant axons have maximal diameters of 1·3 and 0·7 mm. in the anterior nerve-cord. D and E represent small animals with correspondingly small giant axons. The extreme variation shown by the results was quite unexpected and theoretically can be ascribed to two factors: (1) variation in the size and shape of the giant fibre in different animals, even of approximately the same size; (2) variation in the extent of contraction of different body regions during fixation. The following facts are apparent, however. The giant axon has a greater diameter in larger than in smaller animals. Its diameter is maximal in the posterior thorax or anterior abdomen and decreases posteriorly. The diameter of the giant axon increases greatly during the contraction of the animal. It may be concluded that in any study of the conduction properties of this axon, each animal will have to be treated as an independent unit, and the shape and size of its giant axon determined.

A further study was made of the variation in size of the giant fibre relative to the volume of the nerve-cord and the total body volume. This is shown in Text-fig. 7. These figures were obtained by making a series of camera lucida drawings of the parts concerned at several levels, cutting out and weighing the pieces of paper, and multiplying the mean of the weights so obtained for each section of the animal by the length of that section (the method, of course, is based on the assumption that all parts of the animal have the same density and makes no allowance for body fluids). The resultant figures are summarized in Table 1. The giant fibre occupies only a relatively small proportion of the anterior prostomial and oesophageal mass of nervous tissue, viz. 1·1 per cent., while, posterior to this region, it forms 32 per cent. of the



Text-fig. 6. Reconstructions to scale of the nerve-cord and giant axon of five specimens *M. infundibulum* fixed in different states of contraction and expansion: A, extended by cohol anaesthesia; B–E, partially contracted by the fixative. The figures were drawn from aximal horizontal diameters of the structures concerned. The external line in each figure presents the boundary of the nerve-cord, the internal shaded area the giant axon. The nterior thoracic and oesophageal regions have been determined in specimen A only; the maining figures begin at about the level of the fourth setiger. The two lines to the left of ch figure indicate normal lengths of the entire worm when extended and contracted prior death.

TABLE I. *Relative volumes of body, nerve-cord, and giant fibre*

1. Region of prostomium and setigers I and II (containing supra-oesophageal ganglia, oesophageal connectives, and sub-oesophageal ganglion).

Body volume	3,847·6
Nerve-centre volume . . .	130·8
Giant fibre volume . . .	1·5 units

2. Thoracic and abdominal regions (combined results).

Body volume	65,967·6
Nerve-cord volume . . .	665·1
Giant fibre volume . . .	231·3 units

3. Ratios.

A. Prostomium and setigers I and II.

$$\frac{\textit{Anterior nerve centres}}{\text{Body volume (less crown)}} = 0{\cdot}036 = 3{\cdot}6 \text{ per cent.}$$

$$\frac{\textit{Giant fibre}}{\text{Anterior nerve-centres}} = 0{\cdot}011 = 1{\cdot}1 \text{ per cent.}$$

$$\frac{\textit{Giant fibre}}{\text{Body volume (less crown)}} = 0{\cdot}0005 = 0{\cdot}05 \text{ per cent.}$$

B. Thorax and abdomen, posterior to setiger II.

$$\frac{\textit{Nerve-cord}}{\text{Body volume}} = 0{\cdot}0109 = 1{\cdot}1 \text{ per cent.}$$

$$\frac{\textit{Giant fibre}}{\text{Nerve-cord}} = 0{\cdot}319 = 31{\cdot}9 \text{ per cent.}$$

$$\frac{\textit{Giant fibre}}{\text{Body volume}} = 0{\cdot}003 = 0{\cdot}3 \text{ per cent.}$$

C. Entire animal (less branchial crown).

$$\frac{\textit{C.N.S.}}{\text{Body volume}} = 0{\cdot}01 = 1 \text{ per cent.}$$

$$\frac{\textit{Giant fibre}}{\text{C.N.S.}} = 0{\cdot}266 = 26{\cdot}6 \text{ per cent.}$$

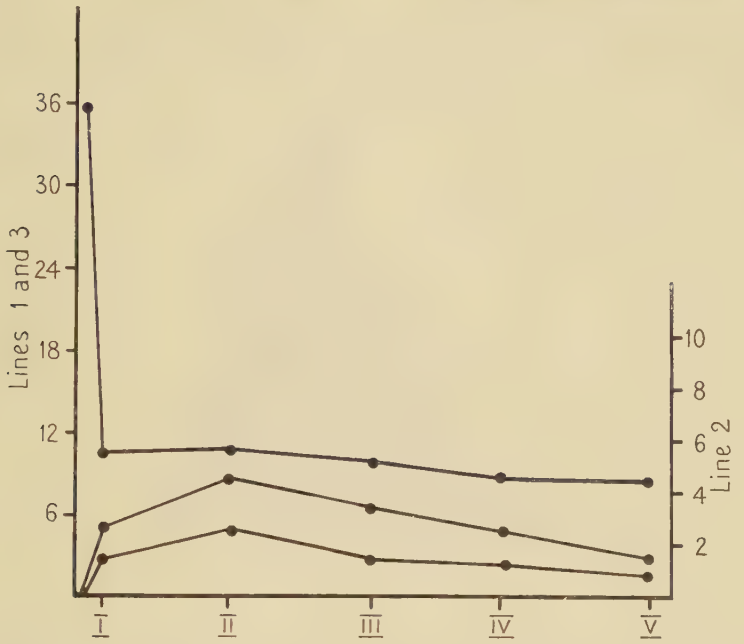
$$\frac{\textit{Giant fibre}}{\text{Body volume}} = 0{\cdot}003 = 0{\cdot}3 \text{ per cent.}$$

volume of the nerve-cord. This single fibre constitutes, therefore, slightly more than 25 per cent. of the entire central nervous system of the animal (exclusive of the branchial crown). The curves shown in Text-fig. 7 bring out two further points: behind the sub-oesophageal ganglion the cord has a nearly constant volume, relative to that of the body; the giant fibre, on the other hand, relative to both cord and body volume, decreases considerably caudally.

(*c*) *Nerve-cells*

Careful examination of serial sections has shown that the giant fibre is connected with nerve-cells throughout its length. The nerve-cells of the cord vary in diameter from about 10μ to 50μ and are elongate or pyriform, the majority being somewhat ellipsoid in shape. They appear to be uniformly unipolar since in no case was more than one cell process seen. In appearance all the cells of the cord are of the same type. The cytoplasm appears finely granular;

there are no distinct neurofibrillae. The nuclei are oval or reniform, about 9μ in diameter, contain a single, basiphilic nucleolus, about 3μ in diameter, and sparse granules or flakes of chromatin. The cell process arises from a



TEXT-FIG. 7. Curves showing the relative volumes of giant axon, nerve-cord, and body of *M. infundibulum*.

Ordinates:

Line 1, $\frac{CNS}{\text{Body volume}} \times 100$

Line 2, $\frac{\textit{Giant fibre}}{\text{CNS}} \times 10$

Line 3, $\frac{\textit{Giant fibre}}{\text{Body volume}} \times 1{,}000$

Abscissae:

I. Prostomium and setigers I and II; II. thorax; III. anterior abdomen; IV. middle abdomen; V. posterior abdomen.

swollen base and contains fine longitudinal fibrillae. The most characteristic feature of the nerve-cells is the presence of numerous vacuoles which are of constant occurrence. Typically each cell contains one or several large vacuoles and a number of smaller ones arranged peripherally about the cell (Pl. 2, fig. 10).

Nissl material may be seen in cells fixed in Carnoy fluid or picro-formol and stained by various standard methods, including toluidine blue and Einarson's gallocyanin. Although groups of nerve-cells in the supra-oesophageal ganglia contain deeply staining masses of tigroid, all the nerve-cells

of the cord contain Nissl material in the form of diffuse and extremely fine granules dispersed throughout the cell. These granules occur in both the perinuclear zone and in the peripheral cytoplasm investing the vacuoles; they are absent from the cell process.

Although all the nerve-cells of the cord have a similar appearance, a non-critical examination of the sections gives the impression that some cells are distinctly larger than the others and have stouter processes and larger nuclei. The possibility therefore arises that different types of nerve-cells may be present in the cord and that they can be distinguished on the basis of size differences rather than of differences in form or content and that among these types may reside the nerve-cells of the giant fibre.

This hypothesis was worth investigating since it has been suggested frequently, and proved in some cases, that the giant fibres of Polychaetes arise from particularly large nerve-cells. Accordingly, cell and nuclear diameters of all nerve-cells were measured in a set of serial transverse sections extending through about one segment in the anterior abdominal nerve-cord. To avoid measuring the same cell twice only those cells were measured that contained nucleoli. In all, about 200 cells were measured and the results are shown in Text-figs. 8 and 9. Maximal and minimal diameters of cells and nucleoli were determined and the means of these two diameters were plotted against frequency. The resulting histograms indicate that the size variation for the cells is a unimodal one. The cells vary in size from 10μ to 50μ; the mean is 25μ (σ 9; se 9·63). Nuclei range in size from 4μ to 16μ; the mean is 9μ (σ 2·5; se 0·17).

Nerve-cells of the giant fibre. The nerve-cells of the giant fibre are of two types, namely, (1) two large cells in the supra-oesophageal ganglia, each of which joins one of the two fibres in this region, and (2) numerous cells which

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PLATE 2. *Myxicola infundibulum*

Fig. 7. T.S. of the ventral half of the nerve-cord. Baker's Ca-formol, frozen section, Sudan black.

Fig. 8. A low-power view of the ventral half of the body by the same technique.

Fig. 9. T.S. of part of the nerve-cord and giant fibre. The photograph is orientated with upper margin corresponding to the dorsal side. Picro-formol, cedarwood oil, paraffin, Holmes's silver, safranin.

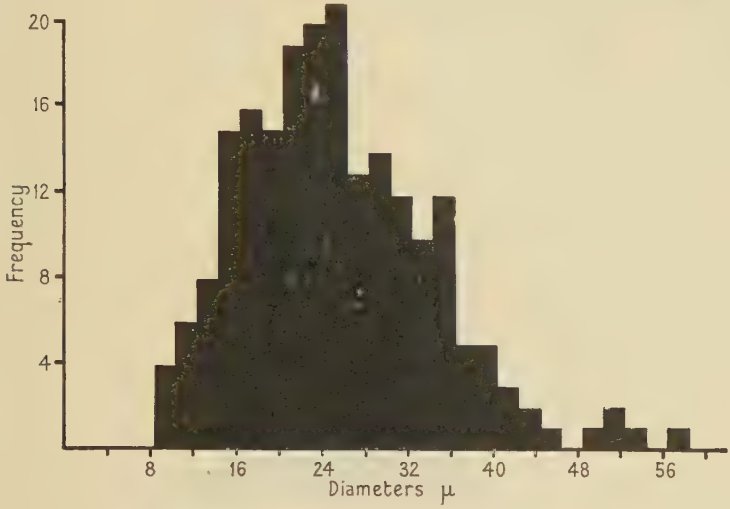
Fig. 10. T.S. of part of the nerve-cord to show a giant fibre cell. Orientation, right-hand side of photograph corresponding to dorsal side. Picro-formol, cedarwood oil, paraffin, Holmes's silver.

Fig. 11. A composite picture built up from three photographs of three successive serial sections. From the ventro-lateral region of the nerve-cord. The result shows a nerve-cell joining a peripheral branch of the giant fibre in a T-shaped junction. The axoplasm is very diffusely and weakly impregnated at the point of fusion between the cell process and the peripheral branch. Picro-formol, cedarwood oil, paraffin, Holmes's silver.

Fig. 12. T.S. of ventro-lateral region of the nerve-cord showing a peripheral branch passing from the giant fibre into the body-wall. This photograph should be compared with fig. 3 in order to understand the difficulty involved in determining whether the peripheral branches are actually continuous with the giant fibre or make synapse with it. The difference in degree of silver impregnation between the giant axon and its branch in this section affords no indication of fusion. Picro-formol, cedarwood oil, paraffin, Holmes's silver.

are distributed along the length of the nerve-cord. The giant fibre cells in the nerve-cord will be considered first.

The above study reveals that the giant fibre cells in the cord have no special peculiarities which distinguish them from the other cells of the cord: they are not giant cells and they have the same nuclear and cytoplasmic features as the other nerve-cells. They are unipolar, often elongate, and highly vacuo-



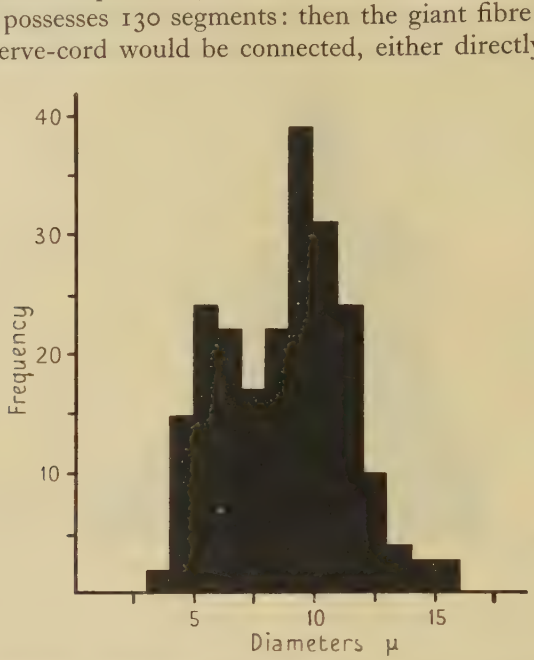
Text-fig. 8. Histogram showing variations in the size of nerve-cells in the nerve-cord of *M. infundibulum*. Population 209. Ordinates, frequency; abscissae, diameters in μ (means of two maximal measurements).

lated, frequently with one or two large vacuoles at each end of the cell. Occasionally they lie ventrally in the nerve-cord, but they are usually seen in a ventro-lateral position. No weight is attached to this observation, however, for it has not been possible to determine all the cellular connexions of the giant fibre and in any series of sections many were certainly overlooked.

The nerve-cells are connected with the giant fibre in two ways: either directly, the cell process joining the giant fibre itself; or indirectly, the cell joining the lateral branches which pass to the periphery of the body. In the first case the cell sends a longer or shorter process through the neuropile of the nerve-cord and this process enters the giant fibre somewhere on its ventral surface after penetrating its fibrous sheath. Frequently, the point of fusion is at the ventro-lateral margin of the giant fibre (Pl. 2, fig. 10). In the second case, a distinct cell process may pass through the nerve-cord to join the lateral branch (Pl. 2, fig. 11); or the cell may lie immediately beside the branch and its cytoplasm and the axoplasm of the lateral branch may fuse with one another over a broad zone of junction.

Because of difficulties in staining all the cell processes and following their course through several serial sections, it has not been possible to enumerate the number of nerve-cells connected with a given length of the giant fibre,

but it is certain that they must be fairly numerous. One to three cells have been seen to fuse with each lateral branch. If there be eight lateral branches per segment, each connected with one nerve-cell; and two additional cells join the giant fibre independently of the lateral branches in each segment; and the animal possesses 130 segments: then the giant fibre throughout its course in the nerve-cord would be connected, either directly or indirectly,



TEXT-FIG. 9. Histogram of size variation of nerve-cell nuclei in the nerve-cord of *M. infundibulum*. Population = 214. Ordinates, frequency; abscissae, diameters in μ (means of two maximal measurements).

but in any case without the interposition of a separating membrane, with 1,300 nerve-cells. It is certain that this figure is a conservative estimate. There are about 300 nerve-cells per segment in the anterior abdomen and thorax of a mature worm and probably about 30,000 in the entire nerve-cord. At least 5 per cent. of the cells present in the nerve-cord, therefore, represent the nerve-cells of the giant fibre and its branches. It is not possible to be more accurate than this, but it is probable that the giant fibre cells are more numerous than the figures given.

The nerve-cells lie among the supporting fibres and cells of the cord and, unlike the giant fibre, lack distinctive investing sheaths. Occasional small cells, with scanty cytoplasm, lie on the nerve-cells like small, ragged tags, but they do not form complete capsules. And although the giant fibre sheath is recurved over the surface of incoming nerve processes and the peripheral branches, it covers them for a short distance only before merging into the surrounding supporting tissue.

The giant fibre cells of the supra-oesophageal ganglia are very characteristic structures, when seen in coronal sections (Pl. 3, fig. 15). There are usually

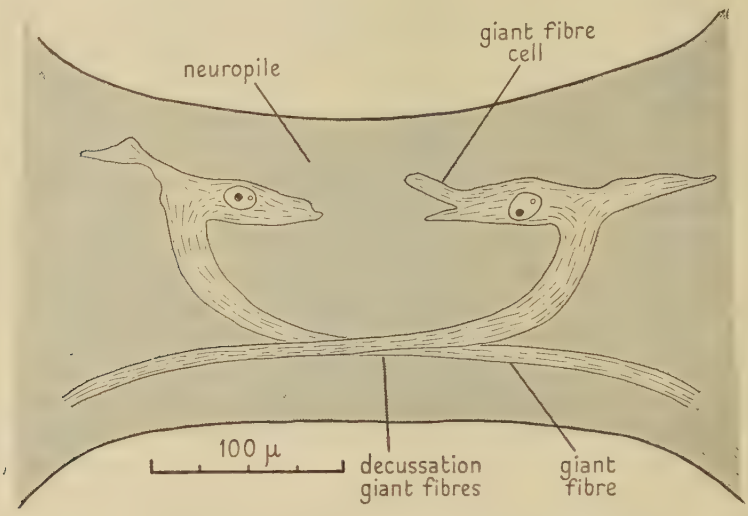
two of them, symmetrically arranged beside one another in a transverse plane, at the extreme ventro-posterior end of the inferior ganglia. Typically these cells are very long and narrow. Each cell extends from the median sagittal plane through the commissural bridge uniting the two inferior ganglia, into the neuropile of the latter. The cell dimensions are about $120\mu \times 30\mu$. The nucleus lies centrally in the cell and is oval or nearly round, $25\mu \times 20\mu$ in diameter. There is a single eccentric nuceolus, 5μ in diameter, and a conspicuous round vacuole, about 4μ in diameter, in the nucleus. The nucleolus stains densely and is basiphilic. The vacuole fails to stain and is surrounded by a rim of dark granules. The cytoplasm consists of a slightly more densely staining central portion closely surrounding the nucleus, and having the appearance of a fine reticulum, the main cytoplasm in the centre of the cell which contains numerous, fine fibrils arranged parallel to the long axis of the cell, and a peripheral, vacuolated zone containing numerous vacuoles of various sizes resembling those of the nerve-cells of the cord. The fibrillae of the cell cytoplasm extend out into the cell process. The cell is apparently unipolar and sends a conspicuously large process from its median end obliquely dorsally and posteriorly to the giant fibre. The process at its origin from the cell has a diameter equal to that of the cell, and twice that of the giant fibre to which it is proceeding; it gradually diminishes from about 30μ to about 15μ before joining the giant axon.

It has been stated above that the giant fibres in the supra-oesophageal ganglia approach each other medially. It is difficult to determine whether they fuse or decussate with one another in transverse sections. They appear to decussate in horizontal longitudinal sections. Careful study and reconstructions of the giant fibre and its cells in sagittal sections show that it is the latter condition which obtains, an arrangement that was quite unexpected and that is completely at variance with the extraordinary amount of interneuronal fusion occurring throughout the remainder of the length of the giant fibre. The two fibres lie in very close apposition in the mid-line, and are separated from one another by their surface membranes only. They clearly twist about each other through an angle of 180°; the fibres remain discrete; they soon separate again and each fibre can be seen to arise as the cell process of a contralateral giant fibre cell (Text-fig. 10; Pl. 1, fig. 2, and Pl. 3, fig. 15). The giant nerve-fibre, therefore, arises from a pair of large nerve-cells in the supra-oesophageal ganglia. The single processes of these two cells decussate with each other in the mid-sagittal plane, and each process passes down the oesophageal connective contralateral to the cell of origin. It is only in the sub-oesophageal ganglion that the two giant fibres fuse together for the first time.

(*d*) *Axoplasm*

When examined in the living animal the giant fibre appears to be completely homogeneous without any indication of microscopic fibrillae or striations. In this regard it is probably significant that Young (1936) failed to find

any clearly marked neurofibrillae in the giant axons of Cephalopods, although definite fibrillae appeared after injury to the nerve-fibre. In preparations fixed and stained by various methods, including both protein precipitants such as picric acid, and non-protein precipitants such as formaldehyde and osmium tetroxide, delicate longitudinal striations can be seen in the axoplasm. Such striations are very fine and are near the limit of optical resolution in the visible spectrum; they are very short, moreover, and are not organized into definite threads that can be followed for any distance. Fine, circularly arranged fibrils



Text-fig. 10. Reconstruction of the giant fibres and their cells in the supra-oesophageal ganglia, based on camera lucida drawings. The cells and the level of decussation are shown in the same plane; actually, the nerve-cells of the giant fibres in this region lie ventrally to the latter.

can be seen also in transverse sections, and these tend to form a definite pattern in that the mat of fine fibrillae lies parallel to the external surface of the axon (Pl. 1, fig. 5). These patterns of fibrillation are particularly well marked near the cut end of an axon and seem to conform to the direction of axoplasmic flow. A somewhat similar configuration was found by Young (1936) in Cephalopod giant axons where fine longitudinal striations could be seen in sections after treatment with various histological reagents. In the case of Polychaetes, Ashworth (1909) stated that the intracellular neurofibrillae of the giant fibre-cells of Halla passed out into the axons, and Cunningham (1888) found that the giant fibres of Sabella were refractory to staining except for fine lines resembling fibres in the axoplasm. Striations of this sort possibly represent a preformed micellar organization of the axoplasm for which evidence has been advanced by Bear, Schmitt, and Young (1937) as the result of birefringence studies.

A very interesting feature of the giant fibre of Myxicola in life is that a

length of this structure may be squeezed out readily from the nerve-cord of the thoracic and anterior abdominal regions. This is best accomplished by cutting open the dorsal surface of the animal, removing the gut, and dissecting free a length of ventral body-wall containing the nerve-cord. When a glass rod is pressed along such a preparation, removed from sea-water, and placed on a glass plate, axoplasm is extruded from the giant axon of several segments. Probably the entire axon is pressed out by this means since its substance is rather viscous and retains clearly the shape that it possesses within the body. In fact strands 3–4 cm. long were obtained on a few occasions by this means and they retained their threadlike form for several minutes before collapsing. It is clear that the axoplasm of this species is much more viscous than that of squid giant fibres (Young, 1934, 1936) and shows little tendency to flow. Whether the viscosity which is revealed very crudely by this means is sufficiently low to permit the great changes in shape of the nerve-fibre that occur in the living animal, or whether there is an alteration in the viscosity of the axoplasm during various phases of activity, must await further investigation.

(*e*) *Sheath*

The giant fibre is surrounded by a distinct sheath which envelops it on all sides. This sheath is about 5μ to 15μ thick and consists largely of a dense meshwork of fine fibrils arranged in a predominantly circular direction about the fibre. Within it are spaced small flattened nuclei at considerable intervals. The fibrils of the sheath are very slender and peripherally are continuous with the fibrous network of the rest of the nerve-cord. They do not appear to be collagenous, they stain poorly with all stains, including connective tissue stains, and they are only slightly darkened by the silver techniques used in this study. The cytoplasm of the supporting cells is probably scanty, but it is impossible to obtain any clear idea of form since the cell-body fails to stain. It is only occasionally that a small tag of protoplasm can be seen about a nucleus. The nuclei are flattened, about $8\mu \times 4\mu \times 2\mu$, with the long axis tangential to the surface of the fibre. Finally, the sheath clings very closely to the giant fibre and there is no indication of any intervening space or cellular layer.

This sheath does not darken when treated with osmium tetroxide, a result which would indicate that myelin, if present, is very scanty in amount. Neither is there any indication of lipines in the sheath when sections are carried through Baker's acid-haematein test. This test does reveal, however, a marked concentration of lipines on or in the peripheral nerves, and in the epidermal cells. The positive blue colour is very striking, and extends into the neuropile of the nerve-cord along the nerve-fibres. Sections stained with Sudan, moreover, give very interesting results (Pl. 2, figs. 7 and 8). In the case of Sudan black, there is a very dense concentration of dye in the subperitoneal tissue and extending into the longitudinal muscles, and lesser concentrations in the epidermal cells, epithelial cells of the gut, in the peripheral nerves, and in

the nerve-cord. Some of the epidermal cells are very heavily coloured and contain dense masses of dye in their peripheral portions. The dye is rather diffusely distributed in the nerve-cord; it is evenly distributed within the cytoplasm of the nerve-cells and there is a slight but clearly distinguishable concentration in the sheath of the giant fibre, in a band about 10μ to 15μ in thickness. The peripheral nerves are deeply coloured, and this coloration clearly extends into the nerve-cord. The peripheral nerve-fibres are very fine and it is not certain where this fatty material is localized; but since the axoplasm of the giant fibre and of its peripheral branches remains negative it is reasonable to assume that the lipoids are concentrated about the peripheral fibres. Somewhat similar results are given by the use of Sudan IV, but the coloration of the nervous system in this case is very light, and gives no indication of a fatty sheath about the giant fibre. Mounted sections were immersed for 1 hour in alcohol-ether and then treated with Sudan black. It was found after this treatment that the dense, subperitoneal deposits of lipoids were largely removed. This tissue probably consists of a layer of fatty cells loaded with triglycerides. The epidermal cells were apparently unaffected by this treatment, while the coloration of the nervous system was considerably reduced and it was no longer possible to distinguish any accumulation of fatty material about the giant fibre. It may be concluded, therefore, that the peripheral nerves are probably thinly myelinated and are invested by deposits of lipoids, in the broad sense, including lipines, and that there is a thin lipoidal sheath about the giant axon (cf. Bear, Schmitt, and Young, 1937).

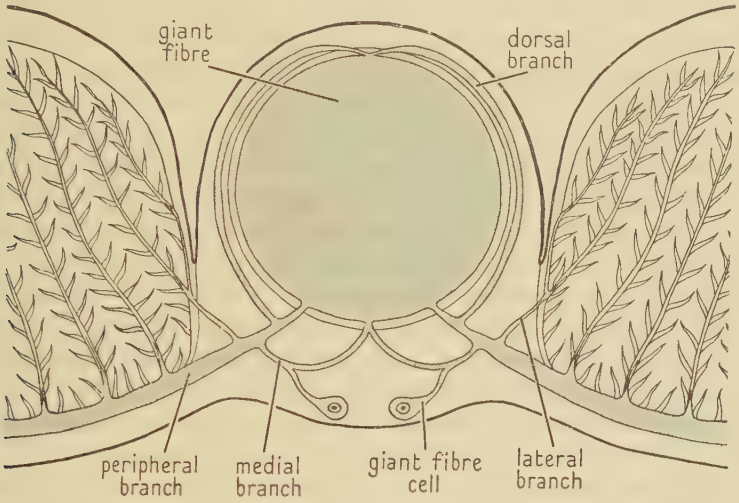
(*f*) *Peripheral branches*

The giant fibre gives off conspicuous peripheral branches along its entire length in the nerve-cord and these branches are distributed to the longitudinal musculature. The branches have typically the following pattern. About four lateral fibres arise from each side of the giant fibre in each segment (Text-fig. 1B). From this position of origin, at a point on the ventro-lateral surface of the giant fibre, they extend obliquely ventrally across the cord to enter the peripheral nerves (Text-fig. 11; Pl. 2, fig. 12). Since there are about twelve peripheral nerves in each segment and only about eight giant fibre branches, some of the peripheral nerves lack these structures. They occur in all of the larger nerves, however. The giant fibre branches divide in a complex manner within the nerve-cord before emerging peripherally. These subdivisions are distributed so as to (1) form additional junctions with the giant fibre, either laterally, medially, or dorsally, (2) unite the giant fibre branches of each side of the nerve-cord, (3) join successive giant fibre branches within the cord, or (4) connect with nerve-cells. Not all of these arrangements have been seen in any one segment or part of a segment, but they have been found sufficiently often to show that the above pattern is typical.

Origin of the peripheral branches. The manner of origin of these efferent branches from the giant fibre shows a great deal of variation and complexity.

These branches not only enter the peripheral nerves but also form an anastomosing web within the nerve-cord, the different branches fusing with one another. Because of their extreme variation in size, the complex courses that they follow and the fact that only the best preparations show clearly their origin from the giant axon, this study has been very difficult.

Usually a peripheral branch of the giant fibre arises by three or more roots from the main fibre, each root rather small, 2μ to 10μ, but more rarely much greater than this, exceptionally as much as 60μ in diameter (Pl. 2, fig. 12).



Text-fig. 11. Diagram of the nerve-cord of *M. infundibulum*, as seen in transverse section. Note dorsal branch of the giant fibre; lateral branch of giant fibre to longitudinal muscle-fibres lying beside the nerve-cord; peripheral branch of the giant fibre. The pennate structures on each side of the nerve-cord represent longitudinal muscles.

The several processes form a root-like aggregation on the ventro-lateral face of the giant axon, fusing with the latter close to one another. Since they tend to be arranged in line, longitudinally, the determination of their arrangement from transverse serial sections entails tedious reconstructions that introduce an element of doubt owing to their complexity. This picture is fully confirmed by horizontal longitudinal sections that reveal this group of branches in a single section.

A superficial examination gives the impression that the peripheral branches are separated by membranes from the giant fibre since there is frequently some suggestion of an intervening structure at the place of junction. This point was carefully examined in serial sections sagittally, frontally, and transversely in material embedded in celloidin and Péterfi's celloidin-paraffin as well as in paraffin sections. Fortunately, such points of junction are frequently very large in some of the thoracic segments (up to 60μ in cross-section) and can be easily studied. In many cases it was clearly apparent that there

is definitely a fusion of substances and the axoplasm of the giant fibre is continuous with that of the peripheral branch over a wide area (Pl. 1, fig. 3).

Giant fibre branches within the nerve-cord. A peripheral branch of the giant axon crosses the neuropile and enters a peripheral nerve. In its passage ventrally through the neuropile it gives off a number of twigs. It is known that some of these twigs have the following arrangement: (1) A process from the peripheral branch extends transversely through the neuropile and forms an additional junction with the giant axon somewhere in the median plane, either singly, or in conjunction with a similar process from the opposite side of the nerve-cord (Pl. 1, fig. 6). (2) A process extends longitudinally through the neuropile to fuse with the next successive peripheral branch of the same side of the nerve-cord (Pl. 1, fig. 4). (3) A process extends dorsally from the main peripheral branch up to even beyond the median dorsal face of the nerve-cord (Pl. 2, fig. 9). Such dorsal processes may expand somewhat as they pass dorsally beside the giant axon and may be quite large, 8μ to 10μ in diameter. An occasional small nerve-cell occurs here and there dorsal to the giant fibre, but it has not been possible to find any connexion between the dorsal processes and such nerve-cells. Moreover, this arrangement appears to be symmetrical in that a dorsal process arises at the same transverse level on each side of the nerve-cord. The processes diminish in size dorsally, approach each other, and terminate on the surface of the giant fibre. The conclusion has been reached that they fuse with one another and with the giant fibre dorsally. (4) Processes of the peripheral branch join nerve-cells in the ventral region of the nerve-cord.

Peripheral distribution of the giant fibre branches. The peripheral branches show considerable size variations in the nerve-cord, but they become more uniform after entering the nerves proceeding to the body-wall (Text-fig. 11; Pl. 2, fig. 12, and Pl. 3, fig. 18). They are about 40$\mu \times 85\mu$ in the thoracic and anterior abdominal nerves of a mature animal and become smaller more posteriorly. Frequently, before emerging from the nerve-cord they give rise to a small lateral twig, which perforates the lateral wall of the nerve-cord above the peripheral nerves proper, and which proceeds directly to the group of longitudinal muscle-fibres which lies immediately beside the nerve-cord, at some distance from the body surface. The peripheral nerve which the lateral branch enters may contain a larger or smaller bundle of small nerve-fibres as well, or may consist of the giant fibre branch only. This branch then proceeds dorsally, between the circular and longitudinal muscle layers, immediately beneath the connective tissue lamella investing the latter, diminishes gradually in diameter, and disappears near the medio-dorsal surface of the animal.

The peripheral nerves are invested by a thin sheath of connective tissue. The branches of the giant fibre may be the sole component of the peripheral nerves, or smaller motor and sensory fibres may accompany them. Bundles of smaller fibres frequently traverse the circular muscle layer and penetrate the epidermis where they lie among the bases of the epidermal cells, but the

branches of the giant fibre maintain a constant position beneath the layer of longitudinal muscles.

The longitudinal musculature of *M. infundibulum* has been described briefly by Claparède (1873) and Johansson (1927). It is very strongly developed and extends the entire length of the animal. The muscle-fibres themselves consist of longitudinal ribbons flattened in transverse section and are arranged along a central axis in a manner that gives the muscle layer a typical pennate appearance when seen in transverse section. The central axes or stalks radiate inwards from the periphery and contain nerve-fibres and a small number of connective tissue fibres which are continuous with the thin sheet of connective tissue underlying the whole longitudinal muscle layer (Text-fig. 11; Pl. 1, fig. 1). The fibres are constructed on the 'nematoid' pattern (cf. Stephenson, 1930), and consist of two closely apposed contractile plates, with a thin intervening layer of sarcoplasm. The peripheral branches of the giant fibre lie directly beneath the layer of longitudinal muscles and over short stretches the intervening connective tissue lamella disappears and the bases of the muscle-fibres rest directly on the nerve-fibres (Text-fig. 11; Pl. 3, fig. 16). However, only a small proportion of the muscle-fibres lies peripherally on the connective tissue lamella underlying the layer of longitudinal musculature; the majority are attached like barbs to the radiating stalks and slender twigs of the peripheral branches of the giant fibre enter these stalks. It has not been possible to follow these secondary branches for any distance into the stalks because they become very slender and disappear from sight. They are about 4μ in diameter where they enter the stalks and soon dwindle to very slender strands. Nervous structures among the muscle-

PLATE 3

All preparations cleared in cedarwood oil and embedded in paraffin wax.

Figs. 13–16 represent *Myxicola infundibulum*, Figs. 17–18 *M. aesthetica*.

Fig. 13. T.S. through the trunk in the anterior abdominal region. The position of the giant fibre in the dorsal part of the nerve-cord and the large volume of the longitudinal muscles are well shown. Formol, cedarwood oil, paraffin, Holmes's silver, light green.

Fig. 14. Horizontal longitudinal section of supra-oesophageal ganglia showing two giant fibres. Helly's, protargol, Heidenhain-azan.

Fig. 15. Another section from the same animal, more ventral to that shown in Fig. 14.

Fig. 16. T.S. through body wall showing branch of giant fibre lying immediately beneath the layer of longitudinal muscles. Helly's, protargol, Heidenhain-azan.

Fig. 17. Horizontal longitudinal section through sub-oesophageal ganglion and anterior nerve-cord, showing giant fibre bifurcating and entering the oesophageal connectives. Picro-formol, Holmes's silver.

Fig. 18. Another section through the nerve-cord further caudad to Fig. 17 in the same animal.

Legend: *c*, nerve-cord; *d.b*, dorsal branch of the giant fibre; *d.g.f*, decussation of two giant fibres in the supra-oesophageal ganglia; *e*, epidermis; *g*, gut; *g.f*, giant fibre; *g.f.c*, nerve-cell of the giant fibre; *g.f.s*, sheath of the giant fibre; *i.l*, inferior lobes of the supra-oesophageal ganglia; *l.b*, longitudinal branch of the giant fibre; *l.m*, longitudinal muscle; *m.b*, median branch of the giant fibre; *n*, neuropil; *n.c*, nerve-cell(s); *n.c.f*, process of giant fibre nerve-cell; *n.s*, sheath of the nerve-cord; *p.b*, peripheral branch of the giant fibre; *p.b.f*, twig of peripheral branch extending into longitudinal musculature; *p.n*, peripheral nerve; *p.n.f*, peripheral nerve-fibres ending in neuropile; *v*, vacuole in nerve-cell; *v.m*, ventral mesentery.

fibres that could be interpreted as terminal expansions, plates, or even branching terminations of nerve-fibres have never been observed. It is possible that the connexion between the nerve- and muscle-fibres is one of simple contact, since the membranes of the two structures are in intimate contact at those points where the nerve-fibres pass beneath the muscle-fibres (Pl. 3, fig. 16).

The innervation of the longitudinal musculature in the anterior thoracic segments is essentially the same as that described above, but a special case arises when one of the halves of the nerve-cord lacks a giant fibre. There is no evidence that the peripheral branches of the giant fibres cross the mid-dorsal line, and it is probable that the longitudinal musculature of each half of the body receives its nerve-supply exclusively from the nerve-cord of that side. However, in cases where the giant fibre is not present in a lateral strand of nerve-cord in the thorax, it has been observed that a peripheral branch of the giant fibre extends a considerable distance longitudinally in the cord to reach the peripheral nerve from the giant fibre. The giant fibre-supply of the longitudinal musculature thus maintains itself constant even when the giant fibre is absent from a section of the nerve-cord.

The giant fibre branches described above are all efferent branches. A systematic study has not been made of afferent fibres connected with the giant axon. Nevertheless, it has been observed that beside the peripheral branches small fibres pass from the ventral surface of the giant fibre to terminate in the neuropile. The peripheral nerves contain a large number of fine sensory fibres which originate in the epidermis and fan out into the neuropile on entering the nerve-cord, many of them lying just below the giant axon. This histological picture would suggest that there is ample opportunity for the giant fibre to be fired directly by impulses entering the nerve-cord via the sensory nerves, contact between afferent neurones and giant axon occurring either on the surface of the latter, or between fine fibres forming synapses in the neuropile.

(*g*) *Effect of cutting the nerve-cord*

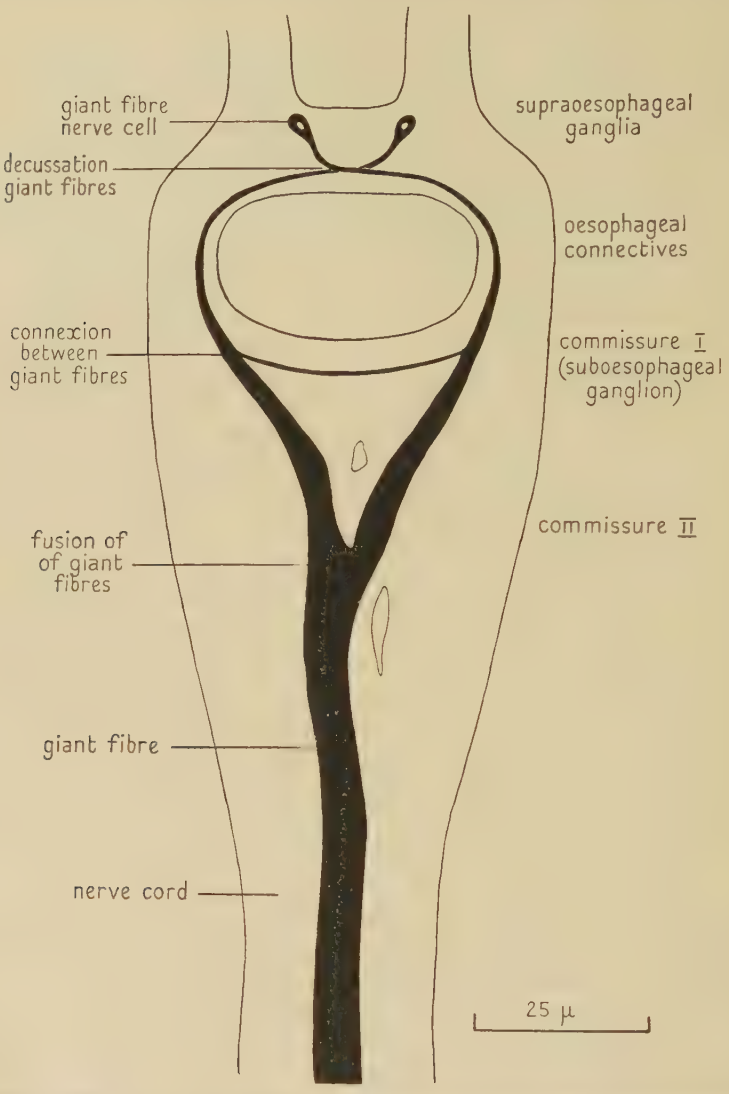
The arrangement of the giant fibre and its cells, and the conception that it represents a vast syncytium extending throughout the length of the body and, indeed, throughout the nervous system and the body-wall, have been based on the examination of preserved material only. Arguing on the basis of our knowledge of nerve-fibres in other animals one would expect that the giant fibre of Myxicola would show one of the following arrangements—it could (1) arise from a single large cell at either the anterior or posterior end of the body; (2) arise from a number of large or small cells at either the anterior or posterior end of the body; (3) arise from many small and/or large cells throughout its length. The picture which has been presented is that of a very large fibre which branches and fuses together several times in the anterior region of the body, which arises from two large cells in the supra-oesophageal ganglia, and which is connected with numerous smaller cells throughout its length. To test the validity of this description, the nerve-cord

and its contained giant fibre were transected in the thoracic region of a number of specimens, the animals were killed after periods ranging from 1 to 16 days, and the nerve-cords were fixed in Bouin's fluid or picro-formol and sectioned for microscopic examination.

There are inherent difficulties in such an experiment owing to the fact that *M. infundibulum*, unlike the allied species *M. aesthetica* and *Sabella pavonina* (Okada, 1932, 1934; Berrill and Mees, 1936, 1936*a*), not only does not regenerate lost tissue, but survives poorly, and begins to show signs of decomposition which spreads from the injured area. In some cases the entire animal was transected in the thorax, and anterior and posterior portions were placed in separate finger-bowls. Heads and thoraces survived well up to at least 30 days, while abdominal portions became completely decomposed after 10 days. The behaviour of the animal provides a useful index of the condition of the giant fibre in injured specimens, since presence or absence of the quick contraction reveals whether or not the axon is healthy. Decomposition affects the giant axon sooner than the rest of the central nervous system. Frequently when the giant fibre response can no longer be elicited, the animal still shows antiperistaltic waves of thickening which extend up to the site of injury, and histological examination of the nerve-cord of such decomposed specimens reveals that the axoplasm of the giant fibre has degenerated into discrete clumps of protein over very considerable stretches, while the remaining neurones of the cord appear normal. This phenomenon can be satisfactorily ascribed to the fact that the giant fibre is a continuous structure and provides an uninterrupted avenue for the transmission of traumatic changes. Nevertheless, it has been possible to obtain several specimens which survived well up to 16 days after section of the nerve-cord, and which showed the giant fibre response throughout that period. In these specimens there was no indication of degeneration of the giant fibre either anterior or posterior to the cut surface. The giant fibre had sealed itself off on either side of the lesion. No regeneration or fusion of the severed ends had occurred; otherwise the fibre, anteriorly and posteriorly, appeared normal.

Information about degeneration times of invertebrate nerve-fibres is scanty, but that which is available indicates that degeneration of the distal portion of an axon separated from its cell-body should occur within 16 days. Although Mönckeberg and Bethe (1899) found that degeneration in the peripheral stump of frog nerves had progressed only a few mm. between 12 and 20 days after injury, Sereni and Young (1932) showed that the course of degeneration in the stellar and mantle nerves of the octopus was very rapid. Granulations had appeared in the axons separated from their cell bodies within 15 hours and after 24 hours the distal axon was full of breakdown products. Also Holmes (1942) found marked degeneration of the smaller fibres in the prawn, 3 days after section of the nerve-cord, although the median giant fibres in the nerve-cord of this animal, fibres which probably are syncytia, failed to degenerate up to 14 days after operation. These experiments indicate that some degeneration of the giant axon of Myxicola should have occurred either

anterior or posterior to the cut region if the fibre has a unicellular origin, or if its nerve-cells are aggregated together in one locus. Since the giant fibre

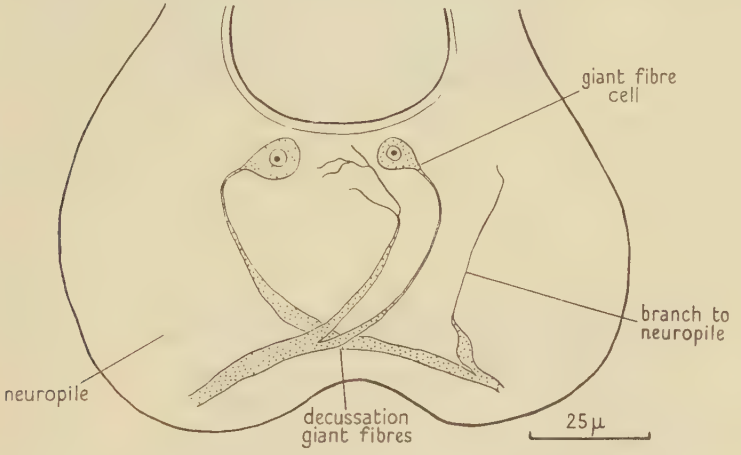


TEXT-FIG. 12. Reconstruction of the central nervous system and giant fibre in the anterior region of *M. aesthetica* (prostomium and setigers I-III). The figure is based on measurements of maximal horizontal diameters in serial transverse segments. Two divisions of the nerve-cord occur in setigers I and II.

is a continuous structure throughout its length the result of this experiment can mean only that it is connected to nerve-cells both anterior and posterior to the thorax. In fact, the giant fibre is a syncytium with nerve-cells throughout its length.

2. *M. aesthetica* Claparède

The nervous system of this species resembles rather closely that of *M. infundibulum*. In general configuration it consists of a short and broad mass of supra-oesophageal ganglia in the prostomium, oesophageal connectives arising from the posterior and ventral surface of these ganglia and joining the sub-oesophageal ganglion in setiger I, and a largely unpaired nerve-cord which extends to the extreme posterior end of the animal (Text-fig. 12; Pl. 3, figs. 17 and 18). The nerve-cord lies in the longitudinal muscle layer and forms a broad flattened mass extending from the sub-epidermal connective



Text-fig. 13. Reconstruction of the two giant fibres and their nerve-cells in the supra-oesophageal ganglia of *M. aesthetica*, based on camera lucida drawings. The outline of the neuropil only is shown; the other nerve-cells of the supra-oesophageal ganglia lie around this region. The nerve-cells of the giant fibres lie slightly ventral as well as anterior to the latter.

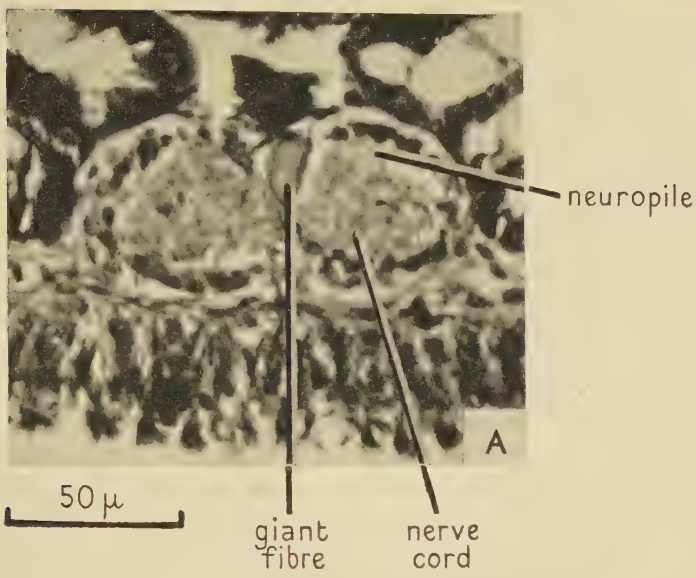
tissue to the coelomic cavity. It is attached laterally to the muscular layer, and dorsally to the ventral mesentery which joins the gut above and which contains a relatively large ventral blood-vessel and a bundle of chloragogen cells. In a specimen 11 mm. long the supra-oesophageal ganglia were 250μ wide, the oesophageal connectives 50μ, sub-oesophageal ganglion 220μ, and the nerve-cord in setiger II 130μ wide. Posteriorly the cord tapers off. It is about 100μ wide in the posterior thorax, diminishes in the posterior abdomen to about 15μ, and gradually expands again in the last few segments to reach a diameter of about 30μ at the posterior end. Although the nerve-cord forms a single strand throughout most of its length, it is bipartite in the anterior thorax as in the larger species of this genus. It divides once in the posterior end of setiger I and again in setiger II, forming a double cord over short intervals of less than one segment in extent. It is covered externally by a thin sheath of connective tissue, overlain by peritoneum on its dorsal face. Nerve-cells are distributed along the entire length of the nerve-cord,

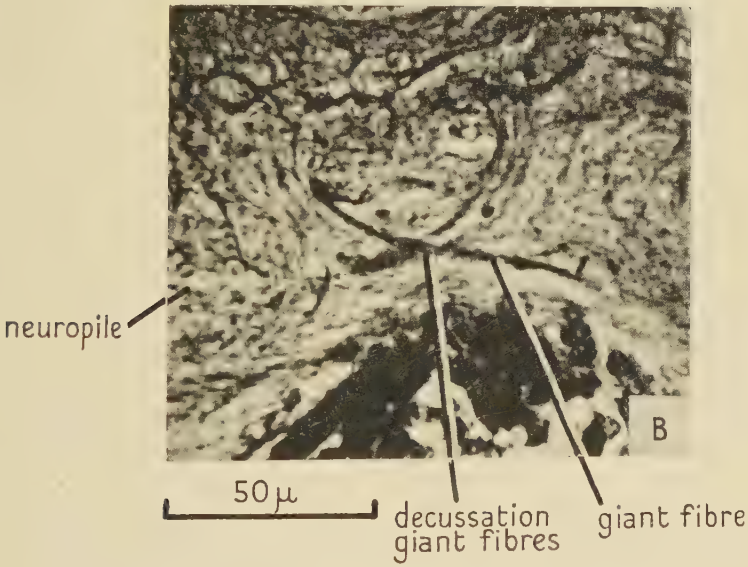
forming a peripheral layer and almost completely investing it, except for a medio-dorsal interval. The interior of the cord contains the neuropile, supporting fibres and cells and, in the superior median region, the giant fibre (Text-fig. 14A).

The giant fibre begins in the supra-oesophageal ganglia. A decussation of two fibres occurs in the posterior and inferior portion of these ganglia, as in *M. infundibulum*, and, after crossing the median sagittal plane, the fibre passes down the contralateral oesophageal connective (Text-figs. 13 and 14B). The two fibres fuse together in the posterior portion of setiger I in the second commissure and the single fibre then extends to the posterior end in the mid-dorsal line. It is relatively smaller than in *M. infundibulum*, when compared with the total volume of the nerve-cord. It has a diameter of about 5μ in the supra-oesophageal ganglia, and increases to 10μ in the oesophageal connectives. It is 40μ broad after fusion in the cord behind the sub-oesophageal ganglion and diminishes gradually to 20μ in the posterior thorax. In the abdomen its diameter decreases from 10μ anteriorly to 2μ posteriorly, with a slight resurgence to 5μ in the posterior swelling of the nerve-cord before terminating in a fine thread at the extreme posterior end. The cord shows regular, segmental variations in diameter, swelling in the middle of each segment and diminishing at intersegmental levels, but although the giant fibre shows some variations in diameter from place to place apart from the trend towards diminution posteriorly, such variations of the giant fibre seem to bear no definite relation to alterations in volume of the nerve-cord itself.

The giant fibre appears to be homogeneous in transverse and longitudinal sections. There is no clearly marked sheath: the fine fibrils which surround the fibre form part of the general supporting network of the cord and show no particular concentration nor peculiarity of arrangement in this area.

Horizontal longitudinal sections of the supra-oesophageal ganglia show that on each side of the region where the giant fibres decussate in the mid-line several branches are given off laterally or anteriorly into the neuropile. Only one of these branches, on each side, has been followed conclusively to its termination and it has been found that it curves anteriorly and ventrally to terminate in a relatively large cell lying centrally on the ventral face of the ganglia. These two large giant fibre cells are symmetrically disposed, one on each side of the mid-line, and are unipolar. They are about 12$\mu \times 16\mu$ in diameter; the nucleus is 8$\mu \times 10\mu$. The cytoplasm is somewhat basiphilic, but stains feebly in all the methods employed, and contains one or a few vacuoles. The nucleus encloses a single nucleolus, 2μ in diameter, and smaller flakes of chromatin. The arrangement of the two giant fibres and their cells in the supra-oesophageal ganglia is very similar, therefore, to that which occurs in *M. infundibulum*. The only significant point of difference is that the giant fibre-cells lie much farther anterior in the supra-oesophageal ganglia of *M. aesthetica* and the giant fibres must extend a long distance anteriorly in the neuropile before reaching them (Text-figs. 13 and 14B).





TEXT-FIG. 14. Photographs of sections of *M. aesthetica*. Picro-formol, cedarwood oil, paraffin, Holmes's silver. 14A. Transverse section of the nerve-cord in the posterior thoracic region. 14B. Horizontal longitudinal section through the level of decussation of the giant fibres in the supra-oesophageal ganglia.

Nerve-cells in the cord stain very poorly with routine stains, such as haematoxylin, and are only slightly impregnated by silver. They vary in size from $8\mu \times 10\mu$ to $12\mu \times 15\mu$. The nuclei range from $4\mu \times 7\mu$ to $8\mu \times 10\mu$. The cytoplasm is finely granular and basiphilic and contains several large vacuoles. The nucleus has a rather scanty chromatin content, and a single nucleolus, 2μ in diameter.

Nerve-fibres in the cord are very small, less than $0{\cdot}5\mu$ in diameter, and this fact, coupled with the weak staining characteristics of the nerve-cells themselves, has rendered it very difficult to determine the cellular connexions of the giant fibre. Nerve-cells at frequent intervals were seen to send a single process towards the giant fibre, but in no case was such a connexion established with certainty. No branches of the giant fibres were seen, although they certainly exist in some form.

VI. Discussion

The giant fibres of Myxicola are unique, even among the Polychaeta in which so many species possess these structures, by virtue of their large size. The maximal diameter of about 1 mm. which the axon occasionally attains in the thoracic region is not exceeded in the animal kingdom, and is equalled only by the giant axons of the stellar nerves of Loligo (Young, 1934, 1936). Comparable fibres of extraordinarily large size relative to that of the animal have been described in the Oligochaete, *Branchiura sowerbyi* Beddard (Stephenson, 1912). Friedländer (1894) had already suggested that the sheath of the giant fibre of *M. infundibulum* was slightly myelinated, and this fact is confirmed by the use of Sudan black which reveals a slight concentration of lipoid about the axon. Among those Invertebrates which possess giant axons there is a great deal of variation in the presence or absence of myelin about these structures. Friedländer (1889), for example, was able to show that the giant fibres of the Annelids Lumbricus and Mastobranchus possessed conspicuous myelin sheaths which he revealed both by treatment with OsO_4 and by examination in polarized light. Ashworth (1909) and Gamble and Ashworth (1900) failed to find a myelin layer about the giant fibres of Halla and Arenicola, respectively, that reduced osmium tetroxide. When the sheath contains only a small amount of lipoid, however, osmium tetroxide is quite ineffective in revealing its presence, as recent studies making use of polarized light have shown. Göthlin (1913) showed that nerve-sheaths which do not normally reveal birefringence due to lipoids may be made to do so by immersion in liquids of suitable refractive index. Bear, Schmitt, and Young (1937) have demonstrated the existence of such a metatropic sheath, that does not stain with osmium tetroxide, in a layer about 4μ thick surrounding the giant axons of the squid. More recently, Taylor (1940) has shown in the earthworm that the sheath of the giant fibres, normally myelotropic and negatively birefringent with regard to its length, may become metatropic at fibre diameters of 10μ–12μ. In *M. infundibulum* the fatty sheath appears to resemble the metatropic sheath of squid axons, except that there is no internal cellular

ayer separating it from the axon itself, as occurs in Cephalopods and Crustacea Bear, *et al.*, 1937). Further studies making use of polarized light should e very informative.

Pumphrey and Young (1938) have calculated that in the case of the squid, ossession of giant axons results in a significant saving of conduction time, factor of obvious survival value when considered in the light of the quick ight reaction which they mediate, and it is interesting to apply the same rgument to Myxicola. In this species the fibre is concerned with a mechanism of quick contraction which causes the animal to retract swiftly into its ube. Ordinary peristaltic waves of thickening, involving successive neurones, re quite slow in their course along the body of this species, and require a ew seconds to traverse the length of the animal. The very fact that the final ommon pathway involved in this quick contraction is a single axon continuous throughout the nerve-cord and with branches penetrating the entire ody-wall obviates all the synaptic delay involved in interneuronal transmission, in the efferent side of the nervous pathway at least. Nicol and Whitteridge (unpublished) have found that the velocity of the nervous impulse n the giant axon of Myxicola is about 11 m. per sec. in the anterior half of n extended animal, a figure that can be taken as a minimal estimation of conduction speed. In the case of Aphrodite, where giant fibres are absent Cunningham, 1888; Rohde, 1887), Jenkins and Carlson (1903) found a nervous impulse to be conducted with a velocity of 54·5 cm. per sec. If these wo figures are employed for purposes of comparison, and only the anterior 7 cm. of animal is considered, a length sufficient to permit withdrawal into ts tube, it follows that possession of a giant axon results in a saving of 87 msec. vhen an impulse traverses this region. The size of the axon is therefore clearly of importance in permitting the animal to retract faster. It has long been known that nerves with thick myelin sheaths conduct faster, other factors being equal, than those without, and recently Sanders and Whitteridge (1946) have been able to substantiate this conclusion quantitatively by taking advantage of the fact that in regeneration of cut mammalian nerves the fibres of the proximal stump have thicker myelin sheaths and smaller axon diameters than normal nerves. Myxicola is obviously another example among the Invertebrates where increase of conduction time has resulted from increase n fibre diameter solely, without concomitant increase in myelin sheath thickness.

Giant nerve-fibres have been described in other Sabellids and there is general agreement that they are particularly well developed in this family, and that they occur in all species (McIntosh, 1877, 1885, 1923; *et al.*). They consist, in general, of very large fibres which extend the entire length of the animal. Two separate patterns can be distinguished, however, which are related to the configuration of the nerve-cord. In the Sabellinae and Fabriciinae, the nerve-cord is double throughout and conforms to the classical 'ladder' pattern, and a large nerve-fibre lies in each half of the nerve-cord. The two fibres are connected together in the commissures of the thoracic

region at least, and extend through the oesophageal connectives into the supra-oesophageal ganglia (Sabella, Branchiomma, Euchone; Claparède, 1873 de St. Joseph, 1894; Brunotte, 1888; Evenkamp, 1931; Thomas, 1940; *et al.*). In the Myxicolinae, on the other hand, a considerable unification of the cord has occurred and this has extended to the giant fibres. The nerve-cord is double for short stretches in the anterior thoracic region only, and is single posterior to that region. In conformity with this arrangement there are two giant fibres in the first few setigers, where they interconnect by transverse anastomoses as in Sabella, while in the posterior thorax and abdomen the fibre is single. The giant fibre also extends into the supra-oesophageal ganglia in this sub-family.

The existence of syncytial giant fibres among different Invertebrates is now a well-recognized phenomenon and Myxicola forms an example at one end of a graded series of such structures which can be traced among the Annelida. At the one extreme there are the giant axons of Halla and Aglaurides, which are unicellular and which extend through a large part of the nerve-cord (Ashworth, 1909). Among the Aphroditids, the giant fibres may be unicellular or a single fibre may arise from the fusion of the processes of a few nerve-cells (Rohde, 1887). The fibres are completely multicellular among the Arenicolidae, where they form an anastomosing syncytium connected with nerve-cells in each segment (Gamble and Ashworth, 1900). Similarly, in the Oligochaeta, some of the giant fibres may anastomose with one another, and their nerve-cells are arranged along the length of the nerve-cord (Friedländer, 1888; Stough, 1926). Stough (loc. cit.) has described transverse, segmentally arranged septa in the giant fibres of both Lumbricids and Nereis. Finally, at the other extreme of this series, lies Myxicola in which the giant fibre is a huge syncytium, continuous throughout its length in the central nervous system, without internal dividing septa, and connected with nerve-cells throughout its entire course.

It is obvious that structures as large as these fibres, and which occupy such a large proportion of the central nervous system, must play an important role in the life of the animal. The most noteworthy feature of the Sabellids is their sedentary mode of existence. All species are tubicolous and retract quickly into their tubes when properly stimulated. This is a mass reaction involving all the longitudinal musculature. Friedländer (1889) stated that Myxicola showed little activity apart from the quick contraction, but this is by no means the only reaction performed by this animal. It also shows slow oscillatory movements leading to burrowing and peristaltic and antiperistaltic waves after suitable stimulation (Nicol, unpublished). Such reactions imply, of course, the existence of a definite, metameric pattern of neurones. They are, however, relatively simple, and there is none of the more complex behavioural patterns such as the sinuous lateral swimming movements which occur in Nereis. It is reasonable to conclude, therefore, that the adoption of a sedentary mode of existence, with attendant abolition of the need for the more complex patterns of behaviour characteristic of free-living forms, has per-

mitted one part of the central nervous system to become highly hypertrophied along with modification of other structural features for a sedentary habit. The resultant modification is a huge, syncytial axon, which causes the animal to act as a unit in quick withdrawal from noxious stimuli. Specialization has led to great simplification of structure and response, but it has also led to restriction in the range of responses possible and, therefore, to sacrifice of plasticity in behaviour patterns.

The writer wishes to thank Professor J. Z. Young for his very helpful criticism during the course of this study; and to thank Professor A. C. Hardy for his encouragement, and other members of his Department for assistance from time to time. Part of this work was carried out at Plymouth, where the writer occupied the Oxford Table. Dr. F. S. Russell and his colleagues at Plymouth have given a great deal of advice and assistance. Grateful acknowledgement is made also of financial aid received from the Canadian Department of Veterans' Affairs and the British Council.

VII. Summary

1. A description is given of the main features of the central nervous system of *Myxicola infundibulum* Rénier.

2. The nerve-cord is double in the first four thoracic segments and single posteriorly. It shows segmental swellings but is not ganglionated in the usual sense in that nerve-cell accumulations are not related directly to such swellings of the cord.

3. A very large axon lies within the dorsal portion of the nerve-cord and extends from the supra-oesophageal ganglia to the posterior end of the animal. It is small in the head ganglia where it passes transversely across the mid-line, increases in diameter in the oesophageal connectives, and expands to very large size, up to 1 mm., in the posterior thorax and anterior abdomen, and gradually tapers off to about 100μ in the posterior body. It shows segmental swellings corresponding to those of the nerve-cord in each segment. It occupies about 27 per cent. of the volume of the central nervous system and 0·3 per cent. of the volume of the animal. The diameter of the fibre increases during contraction of the worm.

4. The giant fibre is a continuous structure throughout its length, without internal dividing membranes or septa. Usually a branch of the giant fibre lies in each half of the nerve-cord in the anterior thoracic segments and these several branches are continuous with one another longitudinally and transversely.

5. The giant fibre is connected with nerve-cells along its entire course; it arises from a pair of cells in the supra-oesophageal ganglia, and receives the processes of many nerve-cells in each segment. There is no difference between the nerve-cells of the giant fibre and the other nerve-cells of the cord.

6. A distinct fibrous sheath invests the giant fibre. A slight concentration of lipoid can be revealed in this sheath by the use of Sudan black.

7. About eight peripheral branches arise from the giant fibre in each segment. They have a complex course in the nerve-cord where they anastomose with one another and receive the processes of nerve-cells. Peripherally, they are distributed to the longitudinal musculature.

8. Specimens surviving 16 days following section of the nerve-cord in the thorax have shown that the giant fibre does not degenerate in front of or behind a cut, thus confirming that it is a multicellular structure connected to nerve-cells in the thorax and abdomen.

9. It is concluded that the giant fibre of *M. infundibulum* is a large syncytial structure, extending throughout the entire central nervous system and the body-wall of the animal.

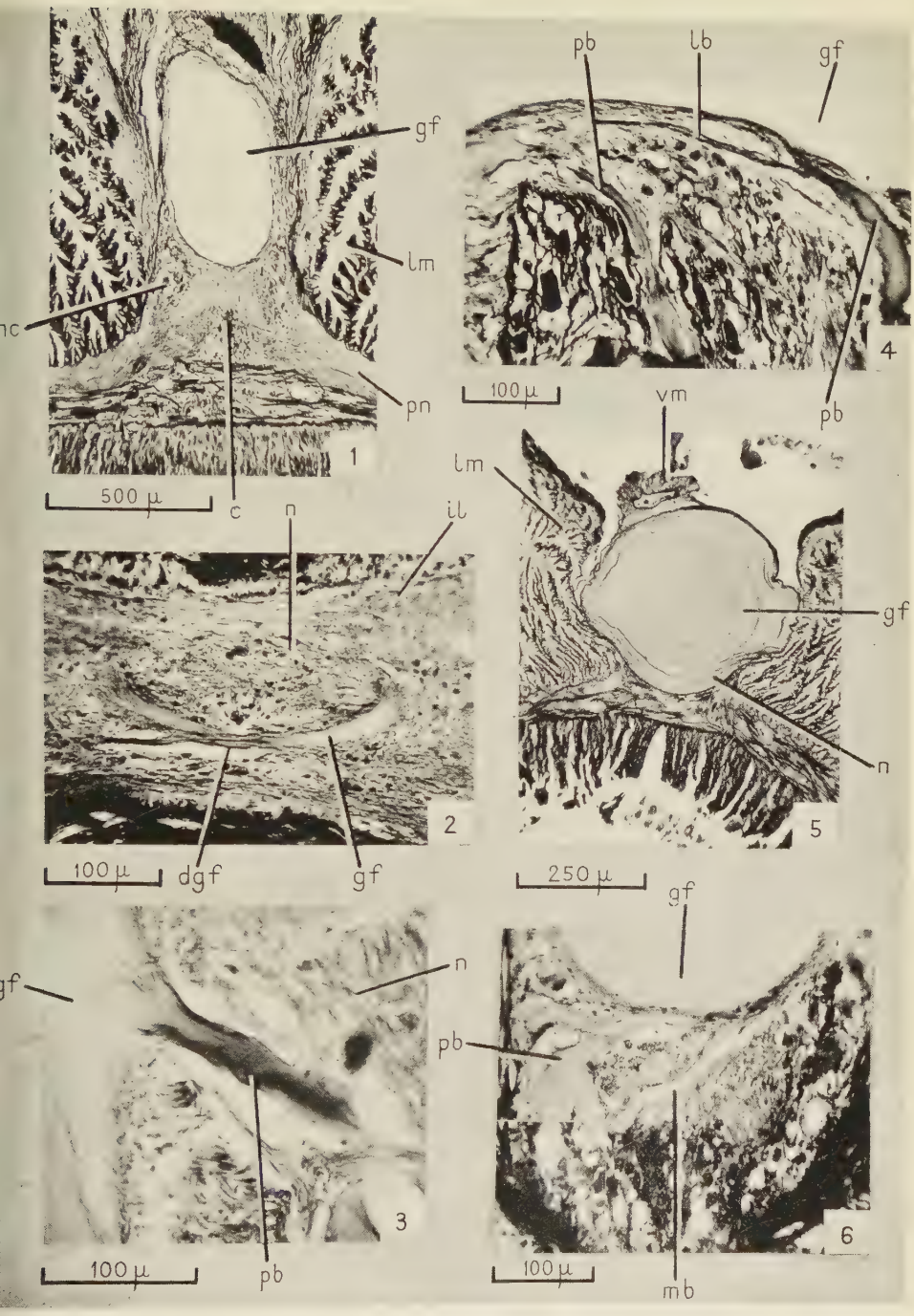
10. The giant fibre system of *M. aesthetica* resembles that of *M. infundibulum*.

11. Some implications of the possession of such a giant axon are discussed. It is suggested that its size, structure, and simplicity lead to rapid conduction and thus effect a considerable saving of reaction time, of considerable value to the species when considered in the light of the quick contraction which it mediates. The adoption of a sedentary mode of existence has permitted this portion of the central nervous system to become developed at the expense of other elements concerned with errant habits.

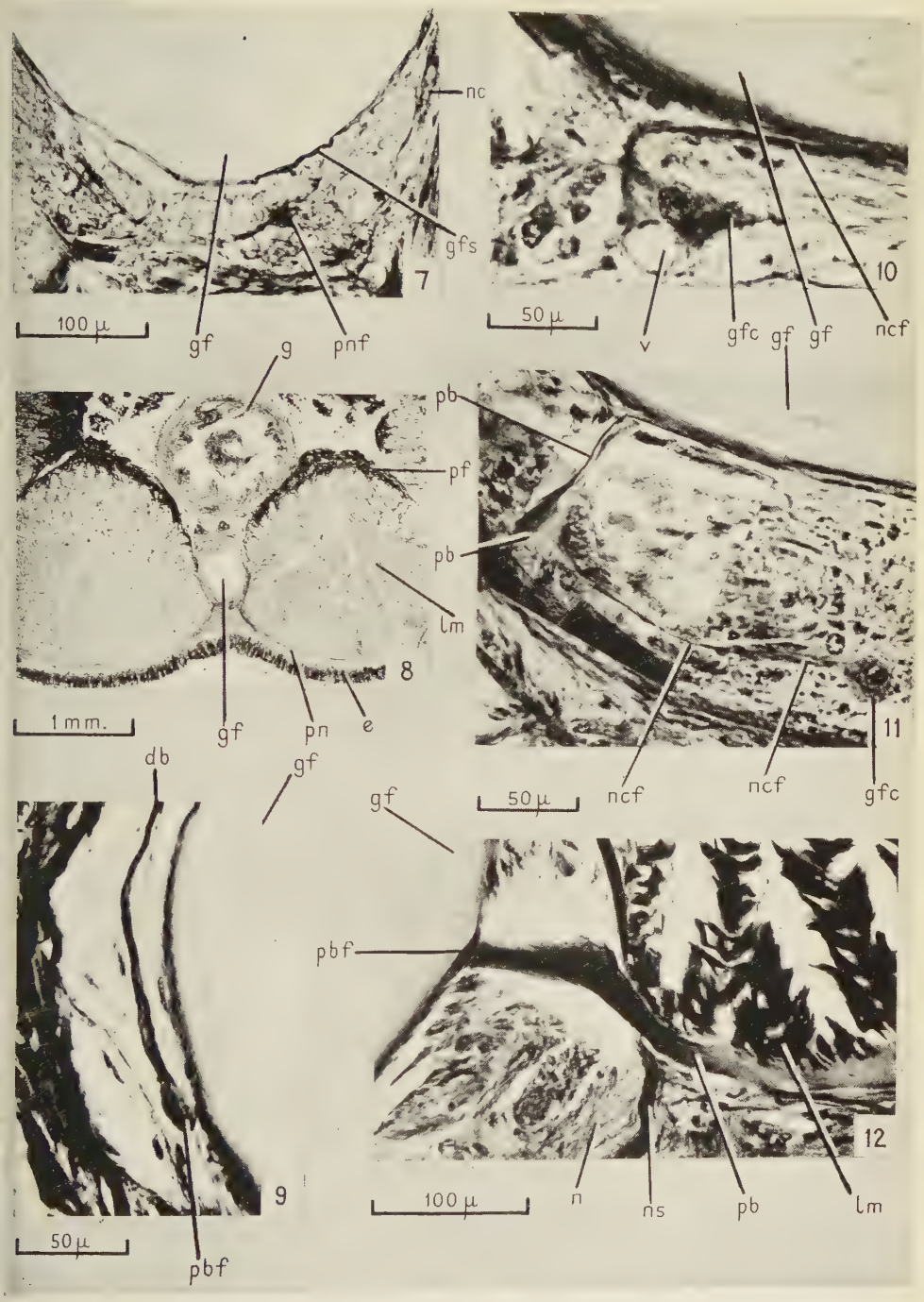
REFERENCES

Ashworth, J. H., 1909. Phil. Trans. Roy. Soc. B, **200**, 427.
Baker, J. R., 1944. Quart. J. micr. Sci., **85**, 1.
—— 1946. Ibid., **87**, 441.
Bear, R. S., Schmitt, F. O., and Young, J. Z., 1937. Proc. Roy. Soc. B, **123**, 496 and 505.
Berrill, N. J., and Mees, D., 1936. J. exp. Zool., **73**, 67.
—— 1936*a*. Ibid., **74**, 61.
Bodian, D., 1936. Anat. Rec., **65**, 89.
—— 1937. Ibid., **69**, 153.
—— 1937*a*. J. comp. Neurol., **68**, 117.
—— 1940. Ibid., **73**, 323.
Boule, L., 1908. Le Névraxe, **9**, 313.
—— 1909. Ibid., **10**, 13.
Bovard, J. F., 1918. Univ. Calif. Publ. Zool., **18**, 135.
Brunotte, C., 1888. Trav. Sta. Zool. Cette. Quoted by Ashworth (1909).
Bullock, T. H., 1944. J. comp. Neurol., **80**, 355.
—— 1945. J. Neurophysiol., **8**, 55.
—— 1945*a*. Quart. J. micr. Sci., **86**, 55.
Carleton, H. M., 1922. Quart. J. micr. Sci., **66**, 501.
Claparède, E., 1861. Mém. Soc. Phys. Hist. Nat. Genève, **16**, 71.
—— 1870. Ibid., **20**, 365.
—— 1873. Ibid., **22**, 1.
Cole, E. C., 1946. Stain Tech., **21**, 63.
Cunningham, J. T., 1888. Ibid., **28**, 239.
Dempsey, E. W., and Wislocki, G. B., 1946. Physiol. Rev., **26**, 1.
Eccles, J. C., Granit, R., and Young, J. Z., 1933. Proc. Physiol. Soc. in J. Physiol., **77**, 23P.
Eisig, H., 1887. Fauna u. Flora Neapel, Monogr., **16**, xxvii + 906.
Evenkamp, H., 1931. Zool. J. Abt. Anat., **53**, 405.
Fedorow, B., 1928. Jb. Morph. mikr. Anat., **12**, 347.
Foley, J. O., Hunt, E. A., and Sackett, W. W., Jr., 1936. Anat. Rec., **64** (Suppl. 3), 65.
Friedländer, B., 1888. Z. wiss. Zool., **47**, 47.

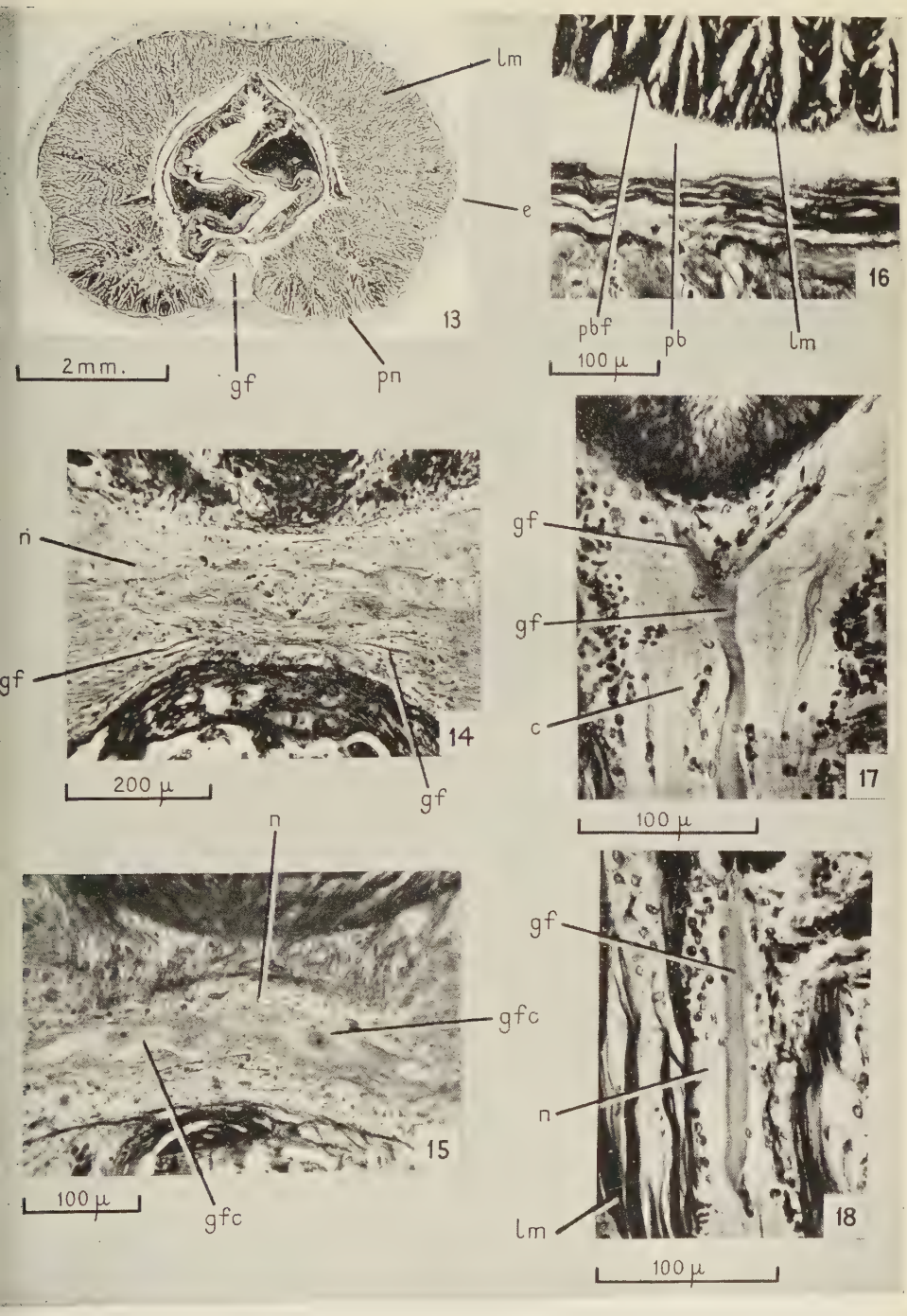
FRIEDLÄNDER, B., 1889. Mitt. Zool. Sta. Neapel, **9**, 205.
—— 1894. Z. wiss. Zool., **58**, 661.
GAMBLE, F. W., and ASHWORTH, J. H., 1900. Quart. J. micr. Sci., **45**, 419.
GÖTHLIN, E. F., 1913. K. svenska Vetenskak. Handl., **51**, 1. Quoted by Schmitt (1936).
HAMAKER, J. I., 1898. Bull. Mus. Comp. Zool. Harvard, **32**, 89.
HANSTRÖM, B., 1928. *Vergleichende Anatomie des Nervensystems der wirbellosen Tiere.* Berlin.
HOLMES, W., 1942. Phil. Trans. Roy. Soc. B, **231**, 293.
—— 1947. *Axon Stain*, in *Recent Advances in Clinical Pathology*, General Editor, S. C. Dyke. London.
JENKINS, O. P., and CARLSON, A. J., 1903. J. comp. Neurol., **13**, 259.
JOHANSSON, K. E., 1927. Zool. Bidrag, **11**, 1.
KOWALSKI, J., 1909. La Cellule, **25**, 289.
KRAWANY, J., 1905. Arb. Zool. Inst. Wien, **15**, 281.
LEWIS, M., 1898. Proc. Amer. Acad. Arts Sci., **33**, 225.
LISON, L., 1936. *Histochimie animale.* Paris.
MCINTOSH, W. C., 1877. Proc. Roy. Soc. Edinb., **9**, 372 (Abstract).
—— 1885. Challenger Reports, **12**, xi+554.
—— 1908–23. *A Monograph of the British Annelids*, Vols. 2, 3, 4. London (Ray Society).
MAXIMOV, A. A., and BLOOM, W., 1944. *A Text-book of Histology*, Fourth Edition. Philadelphia and London.
MEYER, E., 1887. Mitt. Zool. Sta. Neapel, **7**, 592.
—— 1888. Ibid., **8**, 462.
MÖNCKEBERG, G., and BETHE, A., 1899. Arch. mikr. Anat., **54**, 135.
MONTAGU, G., 1808. Trans. Linn. Soc., **9**, 81.
NANSEN, F., 1887. Bergens Mus. Aarsb., 1886, p. 27.
NICOL, J. A. C., and YOUNG, J. Z., 1946. Nature, **158**, 167.
OKADA, Yô K., 1932. Annot. Zool. Jap., **13**, 535.
—— 1934. Bull. Sc. Fr. Belg., **68**, 340.
OWENS, H. B., and BENSLEY, R. R., 1929. Amer. J. Anat., **44**, 79.
PANTIN, C. F. A., 1946. *Notes on Microscopical Technique for Zoologists.* Cambridge.
PRUVOT, G., 1885. Arch. Zool. exp. gén., **3**, 211.
PUMPHREY, R. J., and YOUNG, J. Z., 1938. J. exp. Biol., **15**, 453.
RAMÓN Y CAJAL, S., 1904. Trab. Lab. Invest. Biol. Univ. Madrid, **3**, 277.
RETZIUS, G., 1891. Biol. Unter., Neue Folge, II, 1.
—— 1892. Ibid., III, 1.
ROHDE, E., 1887. Zool. Beiträge, **2**, 1.
RUSHTON, W. A. H., 1945. Proc. Roy. Soc. B, **132**, 423.
—— 1946. Ibid., **133**, 109.
DE ST. JOSEPH, BARON, 1894. Ann. Sci. nat. Zool., **17**, 1.
SANDERS, F. K., and WHITTERIDGE, D., 1946. J. Physiol., **105**, 152.
SCHMITT, F. O., 1936. Cold Spring Harbour Symposium Quant. Biol., **4**, 7.
SERENI, E., and YOUNG, J. Z., 1932. Publ. Staz. Zool. Napoli, **12**, 173.
STEPHENSON, G., 1912. Trans. Roy. Soc. Edinb., **48**, 285.
—— 1930. *The Oligochaeta.* Oxford.
STOUGH, H. B., 1926. J. comp. Neurol., **40**, 409.
—— 1930. Ibid., **50**, 217.
TAYLOR, G. W., 1940. J. cell. comp. Physiol., **15**, 363.
TEN CATE, J., 1938. Arch. Néerland. Physiol., **23**, 136.
THOMAS, J. G., 1940. *Pomatoceros, Sabella and Amphitrite.* L.M.B.C. Mem., **13**.
WAWRZIK, E., 1892. Zool. Beiträge, **3**, 107.
WILSON, D. P., 1936. Quart. J. micr. Sci., **78**, 543.
YOLTON, L. W., 1923. Proc. Nat. Acad. Arts Sci., **9**, 383.
YOUNG, J. Z., 1934. Proc. Physiol. Soc. in J. Physiol., **83**, Pl.
—— 1935. Nature, **135**, 823.
—— 1936. Proc. Roy. Soc. B, **121**, 319.
—— 1938. J. exp. Biol., **15**, 170.
—— 1939. Phil. Trans. Roy. Soc. B, **229**, 465.



J. A. C. NICOL.—PLATE I



J. A. C. NICOL.—PLATE II



J. A. C. NICOL.—PLATE III

Transport of Food through the Alimentary Canals of Aquatic Annelids

BY

JEAN HANSON

(*From the Department of Zoology, Bedford College, University of London*)

DURING an investigation of the blood system in polychaetes of the families Serpulidae and Sabellidae the musculature of the alimentary anal has been studied. In the following species the only muscle coat, which onsists of fibres lying transverse to the main axis, lies outside the blood sinus vhich envelops the canal: *Serpula vermicularis* L., *Hydroides norvegica* Gunnerus), *Vermiliopsis infundibulum* (Philippi), *Pomatoceros triqueter* L., *Protula intestinum* (Lamarck), *Apomatus ampulliferus* Philippi, *Salmacina ncrustans* Claparède, *Spirorbis militaris* (Claparède), *S. corrugatus* (Montagu), *Potamilla* sp. and *Dasychone lucullana* (Delle Chiaje). In *Sabella spallanzanii* Viviani) (Ewer, 1946) there is another layer of circular muscles between the inus and the gut epithelium. This inner muscle coat has also been seen by Evenkamp (1931) in *Laonome kroyeri* and *Euchone papillosa*, and Dr. A. Stock personal communication) has found it in *Sabella pavonina*. In the rest of the iterature on serpulids and sabellids the only circular muscle coat recorded is he one situated in the outer wall of the blood sinus. It has been known for long time (Claparède, 1873) that these muscles contract antiperistaltically, noving the blood forwards in the sinus. In the smaller species it can be seen hat the antiperistaltic contraction waves not only constrict the sinus but requently may also slightly constrict the lumen of the gut. When a contraction wave passes a food bolus the latter is moved for a short distance owards the mouth, but after the passage of the wave quickly returns to its riginal position. Stephenson (1913) in his well-known paper 'On Intestinal Respiration in Annelids' has shown that antiperistaltic contraction of the gut nusculature is not a peculiarity of serpulids and sabellids, but, with very few xceptions, is found in all the many aquatic annelids he examined, whether r not they possess a gut sinus. Where a sinus is absent he postulated that the ntiperistaltic mode of contraction of the gut musculature has been retained uring evolution from ancestors possessing a sinus. Hence, in aquatic annelids in general, the contents of the alimentary canal are not moved along by eristaltic contractions of its wall as they are in other animals, but the activity f the musculature actually tends to hinder their movement towards the anus. The direction of the contraction waves in the extra muscle coat of *Sabella*, *Laonome*, and *Euchone* is not known.

The gut epithelium of aquatic annelids is ciliated, and it is natural to suppose that the cilia are responsible for food transport while the muscles, in

species with a sinus, have a circulatory function. However, Stephenson h stated that, with few exceptions, the cilia in the posterior part of the alimenta canal beat in a postero-anterior direction; and in some species he observ this postero-anterior beating for a long distance in front of the anus. Neve theless, food passes down the gut and faeces leave the anus. Stephenson w especially interested in the function of this 'ascending ciliary current'. H believed it to be respiratory. He did not demonstrate or discuss at any leng how the gut contents move against the current. However, in his account observations on the oligochaete *Aeolosoma hemprichi* he put forward a hypothesis that could be a plausible general explanation of the mechanism food transport in all annelids with antiperistaltic muscle contraction and cil beating towards the mouth. He suggested that the cilia move only the per pheral fluid in the gut and that this ascending peripheral current has as i complement a descending axial current, 'since it is not to be supposed that th postero-anterior current passes through the whole length of the animal an out at the mouth'.

Recently Lindroth (1938) has made some observations which throw dou upon the existence of an ascending ciliary current. He has pointed out th Stephenson did not demonstrate convincingly that the cilia beat towards th mouth. When one examines the abdominal gut of a small intact annelid or can see that the metachronal waves of the ciliated epithelium travel in postero-anterior direction. To Stephenson's list of serpulids and sabelli in which this occurs I can add: *Serpula vermicularis*, *S. lo biancoi* Rio *Hydroides norvegica*, *Vermiliopsis infundibulum*, *Apomatus ampulliferus*, *Sa macina incrustans*, *Spirorbis corrugatus*, *Amphiglena mediterranea* (Leydig), an *Jasmineira candela* (Grube). However, it is well known (Gray, 1928) that many animals the direction of ciliary beat may be the opposite of the directi taken by the metachronal waves, for example, in ctenophores. As Lindroth h pointed out, Stephenson did not discriminate between the metachronal wav and the beating of the cilia. He stated only that: 'The direction of action the cilia is obviously . . . from behind forwards.' Now it is very difficult to di cern the direction of the effective strokes of the cilia in the alimentary cana of serpulids and sabellids: they beat too quickly. In intact specimens o cannot deduce the direction of ciliary beat from the direction of moveme of particles travelling over the surface of the epithelium because, as alrea discussed, the contents of the gut may be moving against the current set u by the cilia. Like Stephenson and Lindroth I have noticed that small partic in the water in the vicinity of the anus may enter the rectum and travel up for a short distance, but they are invariably expelled. Stephenson thought t entrance of particles into the rectum to be additional evidence for the existen of an ascending ciliary current; he attributed the expulsion of the particles an inability on the part of the posterior end of the gut 'to deal with so particles'; 'if possible it avoids receiving them'. Lindroth has shown that t particles are sucked into the rectum during the expansion of the rectum whi follows the initiation of a wave of antiperistaltic contraction of its walls.

Lindroth has seen the direction of beating of individual cilia in the posterior part of the alimentary canal of whole small specimens of *Ammotrypane aulogaster* (Opheliidae). They were beating towards the anus. Moreover, particles near the cilia moved towards the anus, as they also did in the hesionids *Ophiodromus vittatus* and *Castalia punctata* and in an unidentified member of the same family. Lindroth did not consider the possibility that these particles might have been moving under the influence of an axial stream of water returning down the gut. His observations were all made on animals immobilized by narcotization with chloretone; although I know of no evidence that chloretone affects the beating of cilia, it would be desirable to make observations on normal animals. I have found it impossible to distinguish the direction of beating of the cilia in the intact alimentary canals of any of the serpulids and sabellids I have examined. *Salmacina incrustans*, being relatively very transparent, might be thought suitable for this purpose, but the cilia beat too quickly. Preliminary attempts to slow down their rate of movement by lowering the temperature or pH of the surrounding water were unsuccessful. Even when specimens are dying by compression and asphyxiation under a coverslip the cilia beat rapidly up to the moment when their movement entirely ceases.

In larger serpulids and sabellids one can dissect out portions of the wall of the abdominal alimentary canal and study the direction of movement of particles over the exposed ciliated surface. The interpretation of observations is then not complicated by the possibility that the particles are moving against the current set up by the cilia. In this way it has been found that in the following species the cilia beat towards the anus whilst the metachronal waves travel towards the mouth: *Serpula vermicularis*, *Hydroides norvegica*, *Vermiliopsis infundibulum*, *Pomatoceros triqueter*, *Protula intestinum*, and *Sabella spallanzanii*.

Hence it is clear that the cilia in the alimentary canals of these serpulids and sabellids beat in an antero-posterior direction, and that the 'ascending ciliary current' of Stephenson does not exist. Moreover, it seems improbable that it exists in any aquatic annelids. Lindroth (1941) has already discussed the respiration of polychaetes and concluded that the rectum is not a special respiratory organ as Stephenson thought it to be.

In conclusion, it is very probable that the cilia of the alimentary canal in aquatic annelids directly aid transport of its contents. The force exerted by the cilia may be greater than that exerted by the antiperistaltically contracting muscles, but it is probable that food transport is aided by a descending stream of gut fluid initiated in the following manner at the anterior end of the gut. When the mouth is closed or when food is entering the mouth in the course of ciliary feeding, the fluid moved forwards by the antiperistaltic contraction waves is presumably forced to turn back and move down the gut towards the anus. In the same way Stephenson accounted for the transport of food in the gut of *Aeolosoma* in the face of a peripheral antero-posterior stream of water.

The metachronal waves on the ciliated epithelium of the intestine of *Salmacina incrustans* move in a postero-anterior direction, but in the wide part of the alimentary canal situated in the achaetous zone just behind the thorax the waves travel obliquely backwards, the transverse component always appearing clockwise when the animal is viewed from the anterior end. Under the influence of these cilia the boli of mucus and food particles rotate as they move down the intestine and continue to rotate until they have left the anus. A faecal bolus is sometimes arrested half inside and half outside the anus; both parts continue to rotate. In one case a bolus in the rectum was observed rotating while all the cilia in that part of the gut were inactive. The rotation imparts a characteristic spirally sculptured shape to the bolus, and the region of greatest concentration of particles within it takes the form of a spiral. These boli invariably rotate in a direction that appears clockwise when the animal is viewed from the anterior end. This shows that the cilia causing the rotation beat in the same direction as that taken by the metachronal waves. On the contrary, as discussed above, the cilia of the intestine most probably beat in a direction opposite to that taken by the metachronal waves.

Boli rotating in a clockwise direction have also been seen in the abdominal gut of *Serpula vermicularis*, *Pomatoceros triqueter*, and *Spirorbis corrugatus*. Spirally shaped boli have been found in the abdominal gut in *Protula intestinum*, *Sabella spallanzanii*, *Potamilla* sp., and *Dasychone lucullana*. Stephenson (1913) noticed a rotating bolus in the posterior part of the alimentary canal of a specimen of *Spirorbis borealis* and he also found rotating boli in *Aeolosoma hemprichi*, an observation that I have confirmed. Faulkner (1930) noticed occasional rotating food masses in the anterior part of the gut of *Filograna implexa*. It seems very probable that they will be found in other annelids. The rotation presumably facilitates the mixing of enzymes with the food as in *Amphioxus* (Barrington, 1937) and slows up the movement of food through the anterior part of the gut which is probably the region where enzymes are secreted (Nicol, 1930).

Summary

1. In most serpulids and sabellids the only muscle coat in the wall of the alimentary canal lies outside the blood sinus which envelops it. In a few sabellids there is another muscle coat, of unknown function, between the sinus and the gut epithelium.

2. The muscles outside the sinus contract antiperistaltically and tend to hinder the transport of the gut contents towards the anus.

3. The contents of the alimentary canal are transported by its cilia which beat towards the anus. The metachronal waves of the ciliated epithelium travel in a postero-anterior direction. The 'ascending ciliary current' of Stephenson (1913) does not exist.

4. The food boli of serpulids and sabellids rotate as they move down the gut. In *Salmacina incrustans* the rotation is imparted by cilia in the anterior part of the gut.

These observations were made in the Zoological Station of Naples. I wish o record my gratitude to the staff of the Station, to the British Association or the Advancement of Science for the use of its Table, and to the University f London for a grant towards travelling expenses.

REFERENCES

Barrington, E. J. W., 1937. Phil. Trans. B, **228,** 269.
Claparède, E., 1873. Mém. Soc. Phys. Genève, **22,** 1.
Evenkamp, H., 1931. Zool. Jb., Abt. 2, **53,** 405.
Ewer, D. W., 1946. J. Mar. biol. Assoc. U.K., **26,** 426.
Faulkner, G. H., 1930. J. Linn. Soc. (Zool.), **37,** 109.
Gray, J., 1928. *Ciliary Movement.* Cambridge.
Lindroth, A., 1938. Z. vergl. Physiol., **25,** 283.
—— 1941. Ibid., **28,** 485.
Nicol, E. A. T., 1930. Trans. Roy. Soc. Edinb., **56,** 537.
Stephenson, J., 1913. Ibid., **49,** 735.

Variation in the Mouse Adrenal Cortex with Special Reference to the Zona Reticularis and to Brown Degeneration, together with a Discussion of the 'Cell Migration' Theory

BY

I. CHESTER JONES

(From the Department of Zoology, University of Liverpool)

With four Plates and six Text-figures

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1. INTRODUCTION

THIS paper presents the results of a histological study of the adrenal gland of three strains of mice varying in age from 18 days to over 1 year. Two strains are normal (non-cancerous) and one cancerous (the RIII strain of Dobrovolskaia-Zavadskaia). The latter strain shows a peculiar spontaneous degeneration of the adrenal (named brown degeneration by Cramer and Horning, 1937, *a* and *b*). The origin of this degeneration, its variation with strain, sex, and age is given and its suggested relationship to cancer briefly discussed. Brown degeneration is compared with manifestations in the normal adrenal and this, together with an estimate of the amount of cell division in the adrenal cortex, is considered in the light it throws on the normal functioning of the gland with special reference to the 'cell migration' theory (that there is centripetal movement of cortical cells with replenishment from the periphery of the gland and destruction at the medullary border).

2. MATERIAL AND METHODS

The mice used are considered in two main groups, non-cancerous and cancerous, constituting in all 246 animals, 110 males and 136 females.

Group 1. The non-cancerous group is made up of two strains: (*a*) the Wistar albino strain (42 males and 52 females) inbred in this Department for 14 years with no case of spontaneous cancer, and (*b*) a 'piebald' strain (10 males and 19 females) inbred for 5 years with no case of spontaneous cancer.

Group 2. The cancerous group consists of two sub-strains developed from the RIII albino strain of Dobrovolskaia-Zavadskaia (Institut du Radium, Paris). The RIII strain shows a high incidence of mammary cancer in the female but none in the male. The two sub-strains are: (*a*) the CB sub-strain (23 males and 25 females) bred from a male and a female obtained in November 1938 from Dr. Bonser, Leeds University—her Z5/24 and 25; (*b*) the CBB sub-strain (35 males and 40 females) bred from 5 males and 2 females obtained in October 1939 from Dr. Bonser—her Z9/7–11 (males) and Z7/81 and 82 (females).

The mice were killed with chloroform. The chief fixatives used were Zenker, Helly, Susa, 5 per cent. formalin and Bouin, and the stains Ehrlich's haemotoxylin and eosin, Heidenhain's iron haemotoxylin and eosin, Mallory's connective tissue stain, Masson's acid fuchsin aniline blue stain. Sudan III was used for frozen sections after formalin fixation. The majority of the right adrenals were cut longitudinally at 5μ; the remainder and the left adrenals transversely at 5μ. Measurements were made on the right adrenal in all cases.

(i) Measurements (given in μ) were made with a micrometer eye-piece. For estimates of the width of zones the mean is taken from three sections in each gland examined.

(ii) To estimate the size of the gland and the cortex/medulla relationship the 'paper replica' method was used. Only glands cut longitudinally were taken (the few cut transversely were excluded). The median section of each gland was projected ($\times$ 120) on to paper (corrected to a standard weight) and the outline of the cortex and the medulla drawn. The paper replica of the whole section was weighed and that of the section minus the medulla. From this three figures are obtained for each gland.

1. A figure which represents the size of the median longitudinal section.
2. Similarly a figure for the size of the medulla.
3. The amount of cortex in the whole section expressed as a percentage.

These figures are taken to be representative of the whole gland. The method was extended to give the amount of brown degeneration present in advanced conditions. In these cases the outlines of the lobules of degeneration were also drawn and the amount of brown degeneration expressed as a percentage of the whole section.

(iii) The estimate of the amount of cell division in the cortex of a gland was made by examining five sections taken at random from the central bloc of the gland (i.e. sections towards the narrowed ends of the gland were excluded), and the number of mitotic figures in each section counted. The mean of the five sections was corrected for a standard area and the figure termed the 'mitotic index'. The standard area taken was the mean area of the female cortex in the median longitudinal section (as estimated from the

paper replica' weight found in (ii) above). Where an X zone was present the mitoses were not included to give the 'mitotic index' but noted separately; they are not given in this paper as they confirm Whitehead (1933*c*).

Data were, where feasible, treated statistically by the methods of Fisher (1946).

3. Adrenal Size

Three factors are considered: the size of the adrenal, the size of the medulla, and the percentage of the cortex in the whole gland, the figures for which are obtained as given in Section 2 (ii) above. Adult males and adult parous females (i.e. which have had litters) are taken for comparison, that is, adult virgin females, in which the X zone (see Section 4) is an added complication, are excluded. The animals consist of (*a*) the non-cancerous group—33 males, age range 60 days to 375 days; 34 females, 119 to 403 days; (*b*) the cancerous group—35 males, 60 to 546 days; 36 females, 87 to 456 days. No statistically significant difference was found between mice of the same sex in the non-cancerous and the cancerous group and therefore the results for the two groups are given together and summarized in Table 1. The size of the adrenal and the size of the medulla in the male are expressed as a percentage of the female.

Table 1. *Non-cancerous and Cancerous Group Adult Males compared with the Adult Parous Females to show Relative Size of the Adrenal and of the Medulla and the Percentage of the Cortex in the Whole Gland*

(Figures obtained from the median longitudinal section of each gland. S.D.—Standard Deviation; S.E.—Standard Error)

All groups	*No.*	*Size of adrenal*			*Size of medulla*			*Per cent. cortex*		
			S.D.	*S.E.*		*S.D.*	*S.E.*		*S.D.*	*S.E.*
Males .	68	74·30	12·50	1·51	97·47	22·74	2·75	75·64	5·19	0·82
Females .	70	100	24·90	2·97	100	27·12	3·24	82·09	3·54	0·42

From the Table three conclusions are drawn:

1. The gland is bigger in the female than in the male, the difference being about 25 per cent.
2. The medulla is about the same size in males and females. The females show a higher mean, but the difference is not significant.
3. The cortex, expressed as a percentage of the whole gland, is larger in the female than in the male, the difference being about 7 per cent. (This sexual dimorphism can be seen in Pl. I, figs. 1–4.)

The larger adrenal in the female is typical of a number of mammals (review, Parkes, 1945), and has been noted in the mouse by several workers. Only Carlson, Gustaffson, and Möller (1937) give a quantitative estimate. They considered 16 male and 17 female mice all 1 year old and found that the ratio

of the female adrenal weight to the male was as 100 to 47·6 (100; 49·7 corrected for body-weight), that the cortex in the female was 82·66 per cent. of the whole gland, the male 67·55 per cent., and that the medulla was about the same size in males and females. Their results bring out the same points as do mine, the differences in actual figures being due to the variation in method and also probably to the wider range of material in my data.

4. The Normal Adrenal

The description in this section is of the adrenal of the non-cancerous group of mice, but it applies equally to the cancerous group except when in the latter the picture is altered by the supervention of brown degeneration (Sections 5 and 6).

The outer connective tissue capsule, the zona glomerulosa, and the zona fasciculata will not be considered in detail. These layers correspond to the general mammalian pattern and have been described by other workers (Kolmer, 1918; Hett, 1926; Masui and Tamura, 1926; Howard-Miller, 1927; Deanesly, 1928; Whitehead, 1933*b*; Carlson *et al.*, 1937). Pl. 1, figs. 1–5, show the slight variation in general form in these zones although the inner zones differ in the different types of adrenal.

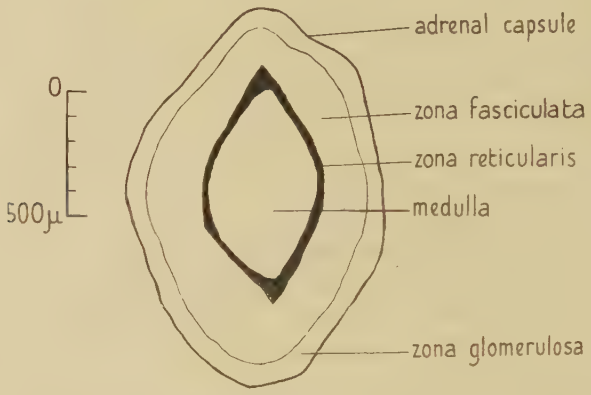
The X Zone. This zone, so designated by Howard-Miller (1927), has been the subject of much research; its function, if any, is still a matter of speculation (see Waring, 1942, for bibliography). My observations on its histological expression are confirmatory of other workers, but a brief review is necessary to obviate any confusion when the zona reticularis and brown degeneration are described. The X zone, the innermost cortical zone surrounding the medulla, is present in immature males and virgin females, and it is absent from mature males and females which have been pregnant. In an undegenerated condition the tissue has a distinctive character, characterized in sections stained with Ehrlich's haemotoxylin and eosin after Bouin by a smooth unbroken appearance, very eosinophil, densely packed and non-vacuolated cytoplasm, the nuclei spherical and of the cortical type. In the male the zone is obvious up to about 28 days, occupying, at the most, 15 per cent. of the cortex in mice of both groups. After this age the zone begins to collapse, the nuclei become pycnotic, and by 38 to 40 days the zone has completely disappeared. In the female the zone does not degenerate at maturity as in the male but increases in size until it forms a broad band around the medulla which may occupy as much as 48 per cent. of the cortex in mice of both groups. My data contain no virgins beyond 3 months, by which age the zone shows some sign of degeneration. It is clear from the literature, however, that the zone gradually disappears with age, usually by 'fatty degeneration' and vacuolization but also by collapse of the cells and pycnosis of the nuclei as in the male (Howard-Miller, 1927, 1939; Deanesly, 1928; Whitehead, 1933*a*). The X zone disappears during the first pregnancy by the twelfth day and often earlier (Tamura, 1926; Howard-Miller, 1927; Deanesly, 1928; Takewaki, 1936).

The Zona Reticularis. The condition of the innermost cortical zone after ne disappearance of the X zone in the adult male and in the primi- or multi- arous female is not so clear. Confusion, which occasionally still persists Kreyberg and Eker, 1939; Parkes, 1945, p. 204), has been caused largely ecause Masui and Tamura (1926) described the special zone, later termed ne X zone, as the zona reticularis. Howard-Miller (1927) states that there s no recognizable zona reticularis in the male. Deanesly (1928) figures a zona eticularis in the male, and in the female, after the disappearance of the X one, considers that a new cortical zone arises which may be transitory. Whitehead (1933, *a* and *b*) doubts the presence of a zona reticularis in the nouse adrenal. Carlson *et al.* (1937) describe a well-developed zone in females year old, with it little developed or absent in coeval males.

In the non-cancerous group parous female, with an age range of 119 to 103 days, a zona reticularis is always present although variable in extent. This applies also to the CBB sub-strain females, age range 115 to 410 days, but not to those females of the CB sub-strain with an advanced condition of brown degeneration (Section 5). The zona reticularis begins where the regular arrangement of the zona fasciculata cells ceases (Pl. I, figs. 3 and 4); there s a transition from the cells of the inner region of the latter zone, which are more deeply eosinophil than those of the outer, to those of the zona reticularis. This zone is characterized by cells of the cortical type in which the cytoplasm s very eosinophil and the cells themselves in stages from slight shrinkage to complete collapse (Pl. II, figs. 6, 7, and 8). The nucleus rarely retains its spherical shape but is in some stage of pycnosis, and in a well-defined zone the nuclei are completely pycnotic. The zone is always vascular and sometimes hyperaemic (Pl. II, fig. 7). Fibrous strands run between groups of cells and are continuous with those of the medullary connective tissue capsule (Pl. II, figs. 6 and 8). The zone is very variable in width not only as between individuals but in the same gland. In some the zone is almost absent from parts of the gland, the zona fasciculata abutting on to the medullary capsule (Pl. II, fig. 9). In others it is as much as 150μ in width, hyperaemic, and made up of completely collapsed cells and pycnotic nuclei staining jet-black with haemotoxylin. Where the gland in transverse section is elliptical, the reticular zone is widest and most prominent at the two narrowed ends of the gland (Text-fig. 1). The zone, in the majority of cases in the non-cancerous group, does not stain with Sudan III—it is 'lipoid' free (Pl. II, fig. 10). In some older mice small lipoid areas are seen in the zona reticularis (Pl. II, fig. 11), and these will be discussed in Section 6 below.

In the mature male, of the non-cancerous group after the disappearance of the X zone, a zona reticularis is largely absent (Pl. I, figs. 1 and 2) and the appearance of the innermost cortical zone is shown in Pl. III, fig. 12. The cells of the inner region of the zona fasciculata, which have eosinophil cytoplasm and spherical nuclei, abut directly on to the medullary capsule and there is no sign of degenerating cells. Even when present the zone is only slightly developed (Pl. III, fig. 13) and rarely completely encircles the medulla,

often showing, in transverse sections, the distribution but not the extent given in Text-fig. 1. Of the 37 males, of the non-cancerous group, ranging from 60 days to 375 days, 20 had no clearly recognizable zona reticularis. In 1 the zone was apparent though usually with the limited distribution noted above. There is no correlation of the presence of a zona reticularis with age. The zone in the male has the same characteristics as described for the female and it does not stain with Sudan III.



TEXT-FIG. 1. Diagram of a median transverse section of the adrenal of a normal parous female. The section shown is roughly elliptical and in these cases the zona reticularis is more prominent at the narrower ends.

In the immature male I cannot make out a zona reticularis although Waring (1935) figures one when the X zone is still present. In the virgin female up to 3 months (beyond which I have no data) the X zone is demarcated clearly from the zona fasciculata, and at this line of demarcation the fasciculata cells have a tendency to splay out and there are a few pycnotic nuclei visible, but this is not extensive enough to be termed a zone.

The Medullary Connective Tissue Capsule. This is a band of connective tissue lying between the cortex and the medulla (Pl. II, figs. 6, 8, 9, 12). According to Whitehead (1933*c*) and Carlson *et al.* (1937) it is more marked in the female than in the male, although Burrows (1945, p. 445) states that it is the male which has the well-developed capsule in comparison with the female.

In the male the medullary capsule appears at about 38 days concomitant with the disappearance of the X zone. It seems likely that the X zone contributes to its formation as Whitehead (1933*a*) and Waring (1935) suggest. From 50 to 60 days it is just discernible as thin strands of connective tissue. From this age onwards (up to 375 days in the non-cancerous group and to 546 days in the cancerous group—the latter showing the same extent and development of the capsule) it is an obvious but thin fibrous layer, of an average thickness of 3μ in section, encircling the medulla as a neat band; there is no thickening with age. The thin capsule, the absence of a prominent

ona reticularis, and the smaller cortex makes the male adrenal recognizably ifferent from the female in section (cf. Pl. 1, figs. 1 and 2 with figs. 3 nd 4).

In the parous female the medullary capsule is generally thick and obvious Pl. II, figs. 6 and 8). It does, however, vary greatly in thickness in the same land, from as much as 80μ to 3μ. An average thickness is therefore difficult o arrive at, but for the 26 mice of the non-cancerous group, age range from 19 to 403 days, it is about 20μ. The capsule is irregular in appearance, ushing into the medulla and more peripherally being continuous with the eticulum of the zona reticularis. The capsule is frequently interrupted by he cortical-medullary blood-vessels.

5. The Origin and Formation of Brown Degeneration

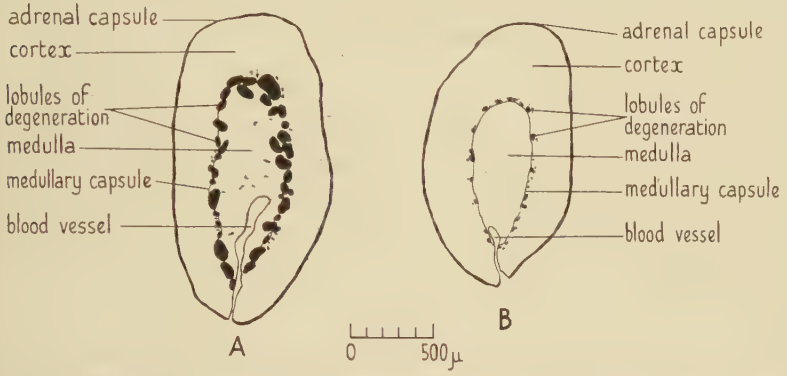
In 1936 Cramer and Horning showed that the prolonged application of estrin to mice produced a degeneration in the adrenal which, in 1937, they amed brown degeneration and noted the spontaneous occurrence of a similar henomenon in the RIII strain. Since then it has been observed, occurring pontaneously, in other—mainly cancerous –strains of mice (Dobrovolskaia-Zavadskaia, 1937; and Zephiroff, 1938; and Pezzini, 1939; Kreyberg and ker, 1939; Blaisdell, Gardner, and Strong, 1941; Bonser, 1941; Kreyberg, 941), and its appearance after oestrogens confirmed (Burrows, 1936; Lacas-agne and Raynaud, 1937; Danner, 1938; Bonser and Robson, 1940). Earlier, lthough not named as such, Kolmer (1918) briefly mentions the same condi-ion in mice of unknown age and origin and Hett (1926) describes its appear-nce in adrenals of mice after intermittent starvation. The physiological ignificance of brown degeneration is not known, but later work has usually escribed it in connexion with mammary cancer, and this will be discussed elow (Section 6). Its site and mode of origin have been the subject of conflict-ng opinions. Burrows (1936) considers that it arises, in oestrin-treated adult nale mice, from a re-established X zone or from cells in that location (1945); Dobrovolskaia-Zavadskaia (1937) that the degeneration is due to phagocytic ction; and Lacassagne and Raynaud (1937) that it is the phagocytes them-elves which have been mobilized and are degenerating. Kreyberg and Eker 1939) state that the origin of the degenerating cells is not always clear but hat the main localization is in the connective tissue between cortex and nedulla. Cramer and Horning (1939*a*) draw a distinction between its origin n oestrin-treated mice and those in which it arises spontaneously. In the ormer they consider that the degeneration arises in the zona reticularis and n the latter its chief site of origin is the medulla. Burrows (1945), however, tates that he has never seen medullary cells involved in the degeneration.

Brown Degeneration in Females of the CB sub-strain. Brown degeneration s present to a greater or less extent in both cancerous and non-cancerous roups. It is, however, the CB females (which have been pregnant) from the ge of 286 days onwards which show the most advanced condition. In this

the degenerative tissue consists of large syncytial masses forming a more c less continuous ring between the cortex and the medulla (Pl. I, fig. 5; Pl. II figs. 14, 15, 16); some masses lie against the medullary capsule, some li embedded within it (Pl. III, fig. 14), and others lie in the periphery of th medulla bounded to the outside by the capsule (Pl. III, fig. 15). In additio small irregular islets are found right in the medulla itself (Pl. III, fig. 16) As many as 60 well-developed lobules can be seen in sections of the gland showing the most advanced condition, and no zona reticularis, as defined fc the normal adrenal (Section 4), is evident. The lobules themselves var greatly in size from as much as $170\mu \times 170\mu \times 205\mu$, as measured in serial sec tion, right down to small but clearly recognizable ones of $12\mu \times 6\mu \times 10\mu$ Each lobule is made up of finely vacuolated cytoplasm with occasional large vacuoles, the cytoplasm being brownish in colour in unstained material an retains its brownish tinge after Ehrlich's haemotoxylin and eosin. It is thi colour which gives the name to the degeneration. The lobules stain afte Heidenhain's iron haemotoxylin and may be termed 'siderophil' (Pl. I, fig. 5) they are orange-red after Sudan III, i.e. 'lipoid' (Pl. III, fig. 16). Scattere throughout the lobule are numerous nuclei, as many as 30 being counted in section. These nuclei are either completely pycnotic or are in some stage o pycnosis (Pl. III, figs. 14 and 15). Some, less degenerate than the rest, ar clearly cortical in type; occasionally a cell outline may be observed abou them (Pl. III, fig. 17).

Stages in the formation of brown degeneration have been found in C females as early as 87 days. The earliest recognizable stages are in the cell of the inner region of the zona fasciculata lying two or three cells from th zona reticularis, this latter zone being discernible in all but the advance condition of degeneration. The first sign is a small but distinct vacuole i such a cell (Pl. IV, fig. 18); further vacuolization occurs (Pl. IV, fig. 19); th cytoplasm becomes less eosinophil and finally does not take the stain at all the nucleus shows pycnosis and the vacuolization spreads to include all th cell (Pl. IV, fig. 20). Such cells stain with Sudan III. Two such cells may b found together usually in the last row of the inner region of the zona fasci culata proper (Pl. IV, fig. 21). Coalescence of degenerating cells takes plac and small lobules are formed with earlier stages of degeneration apparen nearby (Pl. IV, fig. 22). The nuclei are in various stages of pycnosis, the cyto plasm vacuolated with the characteristic brownish tinge now evident, bu the original cortical cells are often obvious. Small lobules may be foun bordering on to the zona reticularis (Pl. IV, fig. 22) or right within this zone surrounded by the degenerated cells and blood spaces characteristic of th normal zone (Pl. IV, fig. 23). Fully formed but small lobules can be see pressed together (Pl. IV, fig. 25) and by coalescence larger lobules ar produced (Pl. IV, fig. 26). These formative stages are all found in the corte itself, occurring right up to the medullary capsule. As the degeneratio becomes more advanced, by further coalescing of lobules, masses are seen i the blood spaces which break the medullary capsule at intervals, and in mor

advanced conditions lobules are sometimes found on the medullary side of the capsule (Pl. III, fig. 15). These lobules, although in the peripheral part of the medulla, are clearly made up of degenerated cortical cells. In the most advanced condition seen the medullary cells themselves degenerate, forming small irregular islets scattered infrequently throughout the medulla. The cytoplasm is vacuolated, brown in colour, and the nuclei degenerating (Pl. IV, fig. 26). The differentiating feature of the medullary islets is that the degeneration remains in areas delimited by the medullary reticular pattern without coalescence, and the rounded, compressed, lobules typical of the cortical degeneration are not formed.



Text-fig. 2. Diagram of the median longitudinal section of the gland showing the most advanced condition of brown degeneration in (*a*) CB females; (*b*) CBB females.

Brown Degeneration in Females of the CBB Sub-strain. In females of this sub-strain of the RIII strain an advanced condition of brown degeneration is not seen. Text-fig. 2, *a* and *b* gives a comparison of the most advanced condition found in the CB and CBB females.

The lobules remain small, the largest seen was $45\mu \times 30\mu \times 50\mu$ measured in serial sections (as against $170\mu \times 170\mu \times 205\mu$ in the CB female), and the median section of the lobule showed 9 nuclei in various stages of pycnosis (as against 30 in the CB female). The stages in the formation of these lobules are the same as described above for the CB female. The very early stages are found in the zona fasciculata, and the lobules themselves are found scattered throughout the zona reticularis (Pl. IV, fig. 23, although of a CB female, shows the position and type of many of the lobules in the CBB female). The reticular zone is readily identifiable in this sub-strain and has the same characteristics and extent as given for the normal female mouse (Section 4). All the lobules are found on the cortical side of the medullary capsule; they may be found against the capsule or embedded within it, but in no case was the degeneration found within the medulla itself. Many of the fully formed lobules in the CBB sub-strain do not show the brown coloration and, remaining unstained after Ehrlich's haemotoxylin and eosin, show up with a grey appearance in the darkly stained zona reticularis.

Brown Degeneration in the Males of the CB and CBB Sub-strains. In th males the lobules are small and not numerous. Their development to thi stage is identical with that given for the females. The lobules lie in the inne region of the zona fasciculata near to or against the medullary capsule or i the zona reticularis when this is present. In no case has the degeneratio been seen in the medulla.

Brown Degeneration in the Non-cancerous Group. The degeneration is nc found in the male, but it occurred in four older female mice (see Section 6) The condition was not advanced, a few small lobules being seen in the zon reticularis (Pl. II, fig. 11) with the early stages of formation apparent a described above for the cancerous group. There is no degeneration in th medullary capsule or in the medulla.

It seems clear that brown degeneration is primarily cortical in origin. I originates in the inner region of the zona fasciculata and lobules are forme from the degenerating cells. These lobules, which increase in size by coale scence, are found throughout the zona reticularis. Cells of this zone, whic have degenerated in the manner typical of the normal mouse adrenal an which are evident in the adrenals of both groups except those CB female which show the most advanced condition of brown degeneration, may them selves become incorporated in the lobules. In later stages the masses o degenerating tissue are found meshed in the strands of the medullary capsule although they do not arise there, and, freed from them, the lobules occur i the peripheral part of the medulla. Only in very advanced conditions are the cells of the medulla involved, and these only to a slight extent, forming smal and easily recognizable islets scattered throughout the medulla. The X zone does not enter into the formation of brown degeneration as this zone has long since disappeared from the mice in which the condition has been described above, i.e. they are adult males and females which have been pregnant. The part played by the reticulo-endothelial system has not been elucidated as its components are displayed principally by vital staining methods (Capell, 1929; Jaffé, 1938; Polak, 1946). It is certain that the lobules are made up of actual cortical cells which have degenerated (and of medullary cells also in some cases) and not of phagocytes. I do not know, however, whether or not the lobules in advanced conditions are added to by members of the reticulo-endothelial system.

6. Brown Degeneration and its Relationship to Mammary Cancer

Cramer and Horning (1936, 1937, *a* and *b*, 1939, *a* and *b*, 1941) and Cramer (1940) maintain that there is a definite connexion between brown degeneration and mammary carcinoma. This is largely based on the fact that mice, after oestrogens, may produce mammary cancer and also show brown degeneration of the adrenal and that the RIII strain of mice, with a high incidence of mammary cancer, have an advanced condition of brown degeneration from the age of 6 months onwards. They point out, however, that

ne degeneration is not in itself the cause of mammary cancer since in the high ancer strains it develops in practically every mouse, male or female, whether ne mammae of these animals are cancerous or not; nor is the development of ancer absolutely dependent on its presence in every case, since in the low-ancer Bagg albino strain and mixed strains mammary cancer may develop vith the adrenal intact. They suggest that the aetiology of mammary ancer may be found in an endocrine imbalance, and the spontaneous develop-nent of brown degeneration in the adrenal medulla is one of the ways in vhich this may be brought about. Dobrovolskaia-Zavadskaia and her co-vorkers (1937, 1938, 1939) conclude that there is some not yet understood onnexion between the two phenomena, and the tendency to brown degenera-ion may be *en rapport* with some endocrine disturbance. Kreyberg and Eker (1939) agree with this general view. Bonser (1941), summarizing the osition, expresses doubt as to the existence of any aetiological relationship etween cancer and degeneration of the adrenal. Blaisdell *et al.* (1941) reach he same conclusion and suggest, in addition, that, as brown degeneration is roduced by the oestrogens and that these too cause hypofunction of the ituitary, brown degeneration may be consequent upon the underfunctioning f the pituitary and especially upon an inadequate amount of adrenocorti-otrophin.

In order to give an estimate of the amount of degeneration present in an drenal a grading scheme was adopted. It is similar to that of Blaisdell *et al.* 1941) except that the higher grades are estimated quantitatively by the nethod given in Section 2. The grades are as follows:

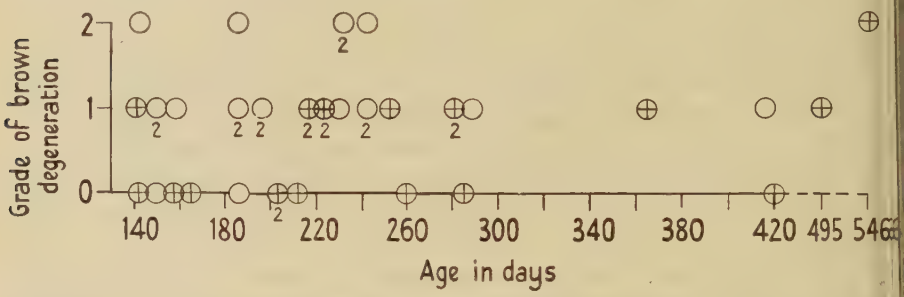
0. No brown degeneration.
1. Early stages visible and a few small lobules (i.e. very little).
2. Obvious lobules scattered sparsely throughout the zona reticularis or, in the case of some males where the zone is absent, in the inner region of the zona fasciculata (i.e. little).
3. The degeneration consists of fully formed lobules with numerous early stages and occupies 1–3 per cent. of the median longitudinal section.
4. The degeneration occupies 3–6 per cent.
5. The degeneration occupies 6–9 per cent.
6. The degeneration occupies 9–12 per cent.

The data for males of the CB and CBB sub-strains are given in Text-fig. 3, nd for females in Text-fig. 4, where in each case the grade of degeneration is lotted against age (from the age at which it first appears in the sub-strains). Those females which had a mammary carcinoma at death (designated by suffix C in Text-fig. 4) are also shown.

CB and CBB males (Text-fig. 3). The age of onset of brown degeneration s about 140 days; only a slight amount of degeneration is shown, the grade never being higher than 2; from 143 to 286 days CB males (with 17 out of 19)

are more inclined to have brown degeneration than coeval CBB males (8 o of 16); of the 5 mice above this age 1 is Grade 0 (420 days), 3 are Grade (365, 418, 495 days), and 1 Grade 2 (546 days).

CB and CBB Females (Text-fig. 4). The degeneration is generally estab lished at 142 days in the CB sub-strain with one early example at 87 day and at 153 days in the CBB. CB females (with 8 out of 22 Grade 3 and abov show a significant difference from the CBB females (28 mice with the grad in no case higher than 2) in the amount of brown degeneration. A fair advanced condition first appears in the CB sub-strain at 186 days and a advanced condition is well established at 286 days and above with one excep tion (Grade 2) at 414 days. Both sub-strains maintain a high incidence o



TEXT-FIG. 3. The grade of brown degeneration in the right adrenal of CB and CBB sub strain males plotted against age (from the age of the first appearance of the degeneration ○ CB males. ⊕ CBB males. Numerical suffix: number of individuals at that point.

cancer formation—8 CB females with mammary cancer out of 9 from 24 to 456 days, and 9 CBB females with mammary cancer out of 19 from 25 to 410 days—but the data are not complete enough to compare incidences There is no correlation between brown degeneration and the appearance o a mammary cancer. Also one mouse (316 days) had a mammary cancer and no brown degeneration. Brown degeneration is generally well established before the usual age of onset of cancer formation.

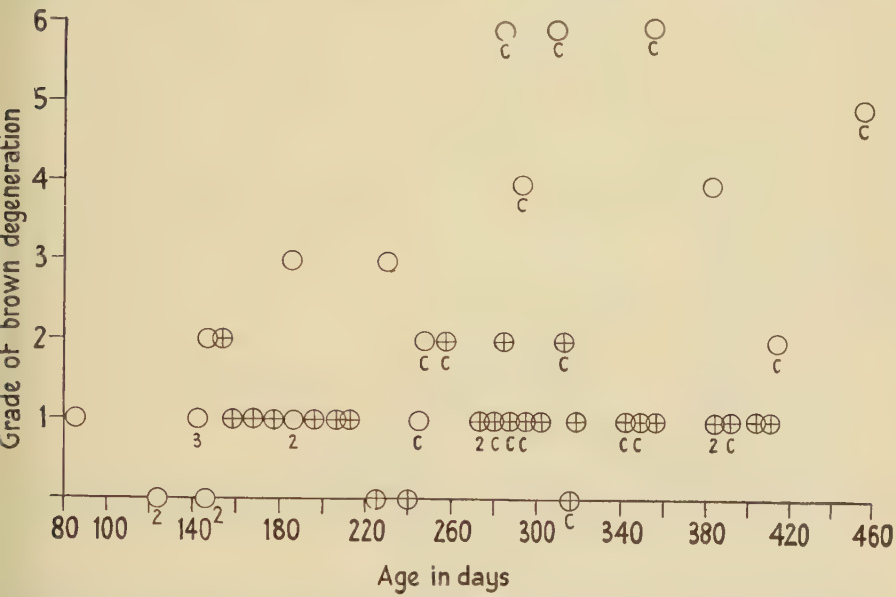
Brown Degeneration in the Non-cancerous Group. The males of this group did not show the degeneration, and it was rare in the females: of the 8 females from 322 days to 403 days, 4 had brown degeneration. These were 1 at 322 days (Grade 1), 1 at 359 days (Grade 2), and 2 at 403 days (Grade 1).

The interest in this section lies in the fact that the new data show that even in the RIII strain a sub-strain has been developed which does not give an advanced condition of brown degeneration yet maintains a high incidence of cancer formation. It appears probable that the existence of some high cancer strains with advanced condition of brown degeneration is coincidental, and that there is no correlation between the two phenomena. In strains of which it is not characteristic it may appear in older females, as shown by those of the non-cancerous group, perhaps as a phenomenon of ageing.

7. Cell Division in the Adrenal Cortex

The method for obtaining the amount of cell division per standard area for each gland—termed the mitotic index—is given in Section 2. The data for the males of the non-cancerous group, the CB sub-strain, and the CBB sub-strain are given in Text-fig. 5, and for the females in Text-fig. 6. In each case the mitotic index for each individual is plotted against age.

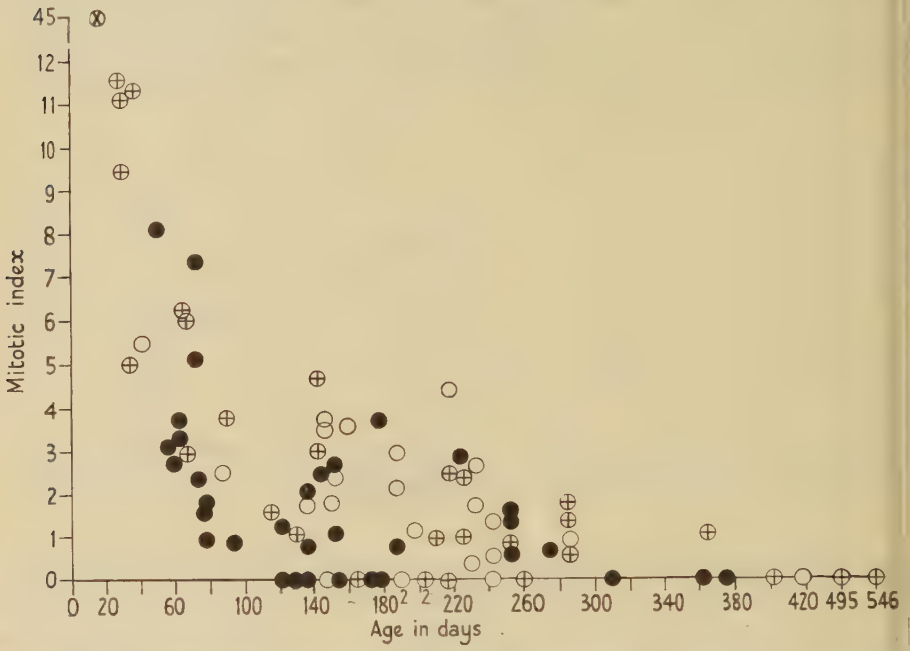
Males (Text-fig. 5). At the youngest age taken, 18 days, the amount of cell division is great (a mean mitotic index of 45 for 5 mice). After this age the



Text-fig. 4. The grade of brown degeneration in the right adrenal of CB and CBB sub-strain parous females plotted against age (from the age of the first appearance of the degeneration) ○ CB females; ⊕ CBB females; suffix C—mammary cancer present at death. Numerical suffix: the number of individuals at that point.

rate falls off rapidly but remains relatively high up to about 60 to 70 days. From 70 to 286 days mitoses are rare and may be regarded as forming part of a Poisson population with a mean number of 1·66 for the 64 mice in the group. For statistical treatment the data were converted to a more normal population by using the conversion formula $\sqrt{(n+\frac{1}{2})}$ where n is the number of mitoses. The variance was found to be about the same over this group. It was found that there is no significant difference between the three sets of males, non-cancerous, CB, and CBB, and that there is no correlation of the amount of cell division with age. Of the 7 mice of 1 year and over, 6 show no cell division and 1 (CBB at 365 days) an index of 1·01. It is probable that there is a falling off in the rate of cell division in the older male mice.

Females (Text-fig. 6). At the younger ages the amount of cell division is great. From 60 to 115 days there is a wide variance from a mitotic index of 1 to 11, and these were not analysed statistically. From 115 to 456 days all the mice in the three sets—non-cancerous, CB, and CBB—have been pregnant (this excludes one primiparous mouse, a CB aged 87 days). These females are considered in two groups, 115 to 359 days and 380 to 456 days, there being no observations between 359 and 380 days. The data are regarded

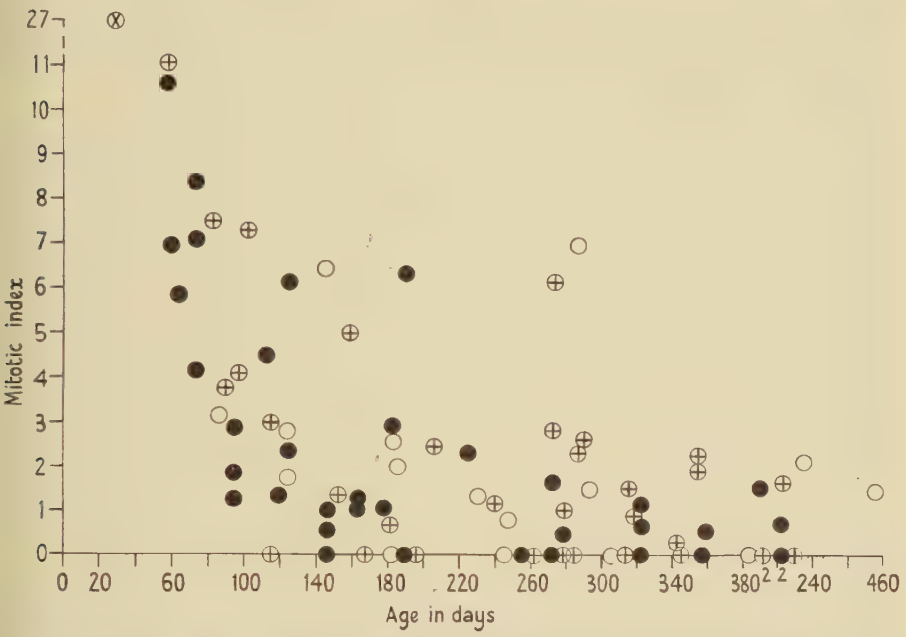


TEXT-FIG. 5. The mitotic index of males plotted against age. ○ CB sub-strain; ⊕ CBB sub-strain; ● non-cancerous group; ⊗ mean of 5 readings (CB, 1; CBB, 2; N–c, 2). Numerical suffix gives the number of individuals at that point.

as being drawn from a Poisson population. The 115–359-days group, of 59 mice, has a mean number of 1·74. Using the conversion formula as given for the males, the group was found to be just about homogeneous although the variation is wide in a few cases. There is no significant difference between the three sets of females—non-cancerous, CB, and CBB—and there is no correlation of mitotic index with age. The second group, 380–456 days consisting of 12 mice, has a mean number of 0·71. Using the conversion formula it was found that this lower mitotic index is significantly different from the 115–359-days group.

Comparing the adult males of all types from 70 to 286 days of age with the parous females from 115 to 359 days it was found that there is no significant difference between the mitotic indices (i.e. 64 males with a mean of 1·66 and 59 females with a mean of 1·74).

There are few mitotic counts for the adrenal of the Mammalia as a whole (see Blumenthal, 1940, 1945) and I am aware of only that of Whitehead's (1933*c*) for the mouse adrenal. He examined three sections from each gland of 107 right and left adrenals from 57 males aged 15 to 336 days and 115 from 61 females aged 15 to 338 days. Up to 60 days his data show the same pattern as mine in both males and females. For male mice aged 85–336 days (45 mice, 87 glands) his mean figure is 1·00 and this is the same order as my mean of 1·66 for 64 mice aged 70–286 days, especially in view of the fact that



Text-fig. 6. The mitotic index of females plotted against age. ○ CB sub-strain; ⊕ CBB sub-strain; ● non-cancerous group; ⊗ mean of 3 readings (CB, 1; CBB, 1; N–c, 1). Numerical suffix gives the number of individuals at that point.

my figures are corrected for a standard area—that of the female cortex—and the male adrenal is smaller than the female (Section 1). For the females aged 85–388 days (46 mice, 91 glands), his mean of 0·42 is much lower than mine of 1·74 for 59 mice aged 115 to 359 days. The correction to a standard area for my data would not account for the difference. There are undoubtedly many unknown factors which influence cortical cell division; it is not clear if the females he took were virgins or had been pregnant; further, Baxter (1946) notes differences between his own data and those of Nathanson and Brues (1941) for the rat, and Blumenthal (1940) between his for the guinea-pig and those of Schmidt and Schmidt (1938) for which methods of sampling by no means account. Whitehead's data do show activity up to the oldest age he examined—for the 8 glands of his male 336-day group the mean is 2·62 and for 8 glands of coeval females the mean is 1·12, and my data show cell division

up to a year in the male and 456 days in the female. There is an indication in my data that the mitotic activity is less over 1 year in both males and females although mitoses are still found in the latter. The function of cell division in the cortex will be considered in the Discussion (Section 8), but it is worth noting here the absence of a clear difference in the mitotic indices of the non-cancerous and cancerous groups, especially as regards the females. For if a causative factor of brown degeneration is the liberation of excess oestrin (Cramer, 1940) it might be expected the CB females would show a significantly high rate of cell division as oestrogen treatment causes a marked increase in the number of mitoses in the rat adrenal cortex (Ellison and Burch, 1936). This might not apply if the action, in cases of the spontaneous occurrence of the degeneration, is via the pituitary (Blaisdell *et al.*, 1941).

The Site of Cell Division. There is no difference between the site of cell division in the non-cancerous and cancerous groups or between males and females. In immature animals mitoses are found throughout the cortex both in the 'permanent' cortex and the X zone. In adult animals the majority of mitoses are found in the inner part of the zona glomerulosa, about 3 to 6 cells in from the outer connective tissue capsule (Pl. IV, fig. 27). The zona glomerulosa, in this region, begins to show the transition to the type of cells found in the outer part of the zona fasciculata. Mitoses are also found, however, anywhere in the zona glomerulosa from this position right up to the outer capsule itself (Pl. IV, fig. 28). Mitoses are occasionally found in the outer part of the zona fasciculata. I have not observed them in the inner region of the zona fasciculata or the zona reticularis. Mitoses are rare in the outer connective tissue capsule and I have not observed one there above 100 days in any group.

Most authors agree that the site of the majority of the cell division in the cortex of the adult mammal is the transition layer consisting of the inner part of the zona glomerulosa and the outer part of the zona fasciculata (Hoerr, 1931; Whitehead, 1933; Bennett, 1940; Blumenthal, 1940; Gruenwald and Konikov, 1944). It seems, from my observations, that the whole of the zona glomerulosa is capable of cell division. I have seen no evidence, in the mouse, for the mechanisms of cell replacement from the adrenal capsule as described for other mammals by Zwemer, Wotton, and Norkus (1938), Salmon and Zwemer (1941), Wotton and Zwemer (1943), Gruenwald and Konikov (1944). These authors do not specifically include the mouse and as the adrenal capsule in this animal is a clear-cut, narrow zone (Pl. IV, fig. 27), with no histological evidence for the transformation of capsular cells to glomerular ones as they figure in other mammals, it is doubtful if their theories would apply to the mouse; Gruenwald and Konikov do in fact exclude mammals with a narrow distinct outer capsule. The germinative role of the capsule seems to be confirmed by the regeneration experiments from enucleated glands (Ingle and Higgins, 1938; Turner, 1939). But, as Ingle and Higgins themselves point out, it is not certain that all glomerular tissue is removed by enucleation and as, in the mouse certainly and in other mammals possibly, cell division of

glomerular cells may take place right against the outer connective tissue capsule, the evidence is not conclusive.

8. Discussion

The 'Cell Migration' or 'Escalator' theory, by which it is considered that the cortical cells are constantly degenerating at the medullary border and as constantly being renewed by multiplication at the periphery and by migration inwards, with cytomorphosis, of the cells so formed, is widely accepted (review up to 1931 by Hoerr; Ivanov, 1932; Zwemer, 1936; Waring and Scott, 1937; Zwemer, Wotton, and Norkus, 1938; Bennett, 1940; Salmon and Zwemer, 1941; Wotton and Zwemer, 1943; Gruenwald and Konikov, 1944; Baxter, 1946; Gruenwald, 1946). The histological appearance of the adrenal of the normal parous female mouse fulfils the two conditions upon which this theory is largely based—that there is cell division at the periphery of the gland, and, at the medullary border, there is a zone of senescence, the zona reticularis. Experimental evidence for the theory is lacking (Calmo and Foster, 1943). The histological facts in regard to the normal adult male adrenal are not so convincing. There is certainly cell division at the periphery of the gland, equal per standard area to that in the female, for a large proportion of adult life. The zona reticularis, however, is at best little developed and often absent. The volume of the cortex is smaller in the male and the cell pressure at the medullary border would not be so great and therefore the zona reticularis would not be expected to be so extensive as in the female. But it is its frequent absence in the male which is surprising if, as the theory requires, there is constant cell destruction in the innermost cortical layer.

If, then, cortical cell division is not concerned, partly or solely, with cell replenishment, it could be evidence of the growth of the gland. Whitehead (1943), discussing the guinea-pig, considered that cortical mitotic activity merely meant that growth was taking place and that the theory of cell migration is not tenable. If this is so it would follow that the mouse adrenal is increasing in size throughout most of its adult life, as my data include mitoses in the male up to 365 days and in the female up to 456 days, Whitehead (1933*c*) shows cell division in the mouse up to 10 months, and, as regards the guinea-pig, Blumenthal (1940, 1945) finds mitoses in animals over 2 years of age. Data to show that the mouse adrenal increases in size throughout the major portion of its life are not available. For the male rat, however, Korenchevsky (1942), while pointing out the relative hypoplasia of organs with age, states that the majority of organs (including the adrenal) continue to grow in the adult, but that towards the middle or end of the period examined (21 to 500 days) the increase in actual weight becomes very slow or may even be stationary. So that the possibility that mitoses in the adult adrenal cortex are concerned with growth is not completely excluded.

This naturally leads to an examination of the alternative to the cell migration theory. By this alternative theory, which I will call for convenience the 'zonal' theory, the zona glomerulosa, the zona fasciculata, and the zona

reticularis are regarded as discrete layers both in structure and function. The functional separation of the first two zones is based on the results following hypophysectomy and ascribes the secretion of the carbohydrate metabolism regulating hormone to the zona fasciculata—which in the cell migration theory is regarded as the site of all cortical hormone production (e.g. Bennett, 1940) —and of the 'salt' regulating hormone to the zona glomerulosa (Swann, 1940; Sarason, 1943; Deane and Greep, 1946). The zona reticularis is considered to be associated with reproduction (review by Vaccarezza, 1946; Blackmann, 1946). There is considered to be no centripetal replenishment of these distinct zones from a germinative layer, but there may be local degeneration and regeneration in each zone (Tonutti, 1941, 1942, *a* and *b*; Vaccarezza, 1946). Deane and Greep consider that the mitoses observed in the inner margin of the zona glomerulosa may only be the regenerative region of the zona fasciculata and, in contrast, the cells of the zona glomerulosa may die locally and be replaced from the capsule. The zonal theory would find support, histologically, therefore, in evidence of cell division throughout the cortex and in the frequent presence of degenerating cells in zones besides the reticular, but such evidence is not forthcoming from the mouse adrenal.

The spontaneous degeneration of the adrenal of the RIII mice is of significance in the consideration of these two theories. For it is difficult to conceive of the advanced condition of brown degeneration, as seen in the CB female, arising except by the inward movement of cortical cells. It has been shown to be a purely cortical phenomenon in its initial stages. Its origin is in the cells of the inner region of the zona fasciculata. It appears as lobules in the zona reticularis which increase in size by coalescence; then in more advanced conditions the lobules are emeshed in the strands of the medullary capsule and freed from them in the medulla itself, still clearly made up of degenerated cortical cells. These facts give a very telling histological picture in favour of centripetal movement of cortical cells.

On the whole the balance of the histological evidence lies in favour of the classical cell-migration theory. The condition of the male mouse adrenal, in both the cancerous and non-cancerous groups, is, however, a challenge which is only partly met by the fact that the volume of the cortex is smaller than in the female. The resolution of the problem must await more experimental evidence.

A question remains in regard to brown degeneration. The factor causing this type of degeneration has not been elucidated, but if the cell-migration theory is accepted it is only another mechanism by which, in any case, the cells of the adrenal cortex degenerate as they reach the innermost cortical layer. Why, however, should there be accumulation of this degenerated material?—and this is a pertinent question, whether the zonal or the cell migration theory be the correct one. In the normal adrenal it is supposed that the dead cells of the zona reticularis are removed—as Hoerr (in Maximov and Bloom, 1946, p. 322) says: 'The cells of the cortex appear to be degenerating and dying continually in the zona reticularis. The debris is removed

mainly by the macrophages which are always present.' The reticulo-endothelial system is the obvious agent by which brown degeneration should be removed and its non-removal may therefore indicate that this system is not so efficient in the CB female, and, to a less extent, in the cancerous group as a whole. It is possible, further, that, as brown degeneration appears in older non-cancerous mice, loss of efficiency is normally a function of age. This is only a tentative hypothesis, but it is interesting to note that Jones (1947), working in this Department, found that haemosiderin accumulation in the uterus was removed by macrophages much less quickly in the RIII strain than in normal mice.

I am very grateful to Mrs. R. C. Bisbee for help and criticism, and to Mr. R. Plackett for aid with statistical methods.

9. Summary

1. The adrenals of two groups of mice, ranging in age from 18 days to over 1 year, were examined. One group is non-cancerous (52 males and 71 females); the other the RIII cancerous strain from which two sub-strains have been developed; (i) the CB sub-strain (23 males and 25 females); (ii) the CBB sub-strain (35 males and 40 females).

2. In the adults of both groups the parous female adrenal is bigger than the male by about 25 per cent.; the medulla is about the same size in males and females; in the female the cortex is 82·09 per cent. of the whole gland, in the male 75·64 per cent.

3. In the parous non-cancerous female, the zona reticularis is always present; in the adult male it is little developed and often absent.

4. Brown degeneration, found in the adrenal of the cancerous group, arises in the cells of the inner zona fasciculata; the subsequent formation of the typical lobules is traced. The medulla is involved in advanced conditions.

5. CB females show an advanced condition of brown degeneration, CBB females do not. Both maintain a high incidence of mammary carcinoma. Four older females of the non-cancerous group have a slight amount of brown degeneration. Cancerous males have little degeneration and non-cancerous males none.

6. The mitotic count per standard area in the cortex is given. There is no significant difference between the non-cancerous and cancerous groups or between adult males and parous females under 1 year of age. Over 1 year there is an indication that the amount of cell division falls off, although in the female mitoses are still found up to 456 days. Mitoses occur all through the zona glomerulosa with the majority towards the inner border.

7. The histological evidence is, on the whole, in favour of the 'cell-migration' theory (the centripetal movement of cortical cells with replenishment from the periphery and destruction at the medullary border). It is suggested that brown degeneration is a variation of the way in which cortical cells

normally degenerate; but the causative factor is not known; it has no direct correlation with mammary cancer, and its occurrence as characteristic of certain cancerous strains may only be coincidental.

10. REFERENCES

Baxter, J. S., 1946. J. Anat., Lond., **180,** 139.
Bennett, H. S., 1940. Amer. J. Anat., **67,** 151.
Blackmann, S. S., 1946. Bull. Johns Hopkins Hosp., **78,** 180.
Blaisdell, J. S., Gardner, W. U., and Strong, L., 1941. Cancer Res., **1,** 283.
Blumenthal, H. T., 1940. Endocrinology, **27,** 477.
—— 1945. Arch. Pat., **40,** 264.
Bonser, G. M., 1941. Proc. 7th Int. Cong. Genet. (1939), 80.
—— and Robson, J. M., 1940. J. Path. and Bact., **51,** 9.
Burrows, H., 1936. J. Path. and Bact., **43,** 121.
—— 1945. *Biological Actions of Sex Hormones.* Cambridge U.P.
Calmo, I., and Foster, C. L., 1943. Nature, Lond., **152,** 536.
Capell, D. F., 1929. J. Path. and Bact., **32,** 595.
Carlson, H., Gustaffson, B., and Möller, K., 1937. Upsala Läk. Forhandl., **1,** 49.
Cramer, W., 1940. Amer. J. Cancer, **38,** 463.
—— and Horning, E. S., 1936. Lancet, Lond., **230,** 237.
—— —— 1937*a*. Nature, Lond., **139,** 196.
—— —— 1937*b*. J. Path. and Bact., **44,** 633.
—— —— 1939*a*. Amer. J. Cancer, **37,** 343.
—— —— 1939*b*. Lancet, Lond., **236,** 192.
—— —— 1941. Proc. 7th Int. Cong. Genet. (1939), 91.
Danner, M., 1938. Klin. Wchns., **17,** 658.
Deane, H. W., and Greep, R. O., 1946. Amer. J. Anat., **79,** 117.
Deanesly, R., 1928. Proc. Roy. Soc. B, **103,** 523.
Dobrovolskaia-Zavadskaia, N., 1937. C.R. Soc. Biol., **125,** 877.
—— and Zephiroff, P., 1938. C.R. Soc. Biol., **128,** 971.
—— and Pezzini, Z. M., 1939. C.R. Soc. Biol., **131,** 240.
Ellison, E. T., and Burch, J. C., 1936. Endocrinology, **20,** 746.
Fisher, R. A., 1946. *Statistical Methods for Research Workers,* 10th ed. London (Oliver and Boyd).
Gruenwald, P., 1946. Anat. Rec., **95,** 391.
—— and Konikov, W. M., 1944. Anat. Rec., **89,** 1.
Hett, J., 1926. Z. f. mik. anat. Forsch., **7,** 403.
Hoerr, N., 1931. Amer. J. Anat., **40,** 251.
Howard-Miller, E., 1927. Amer. J. Anat., **40,** 25.
—— 1939. Amer. J. Anat., **65,** 105.
Ingle, D. J., and Higgins, G. M., 1938. Endocrinology, **22,** 458.
Ivanov, M. J., 1932. Arch. Russ. d'anat. d'hist. d'embryol., **11,** 219.
Jaffé, R. H., 1938. *Handbook of Hematology,* vol. ii (Chap. 15), ed. H. Downey. London (Hamish Hamilton).
Jones, V. E., 1947. Quart. J. micr. Sci. (in the press).
Kolmer, W., 1918. Arch. mikr. Anat., **91,** 51.
Korenchevsky, V., 1942. J. Path. and Bact., **54,** 13.
Kreyberg, L., 1941. Proc. 7th Int. Cong. Genet. (1939), 178.
—— and Eker, R., 1939. Avhand. ut. av det Nor. Viden. Akad., Oslo I. Mat. Nat., **3,** 3.
Lacassagne, A., and Raynaud, A., 1937. C.R. Soc. Biol., **124,** 1183.
Masui, K., and Tamura, Y., 1926. J. Coll. Agric. Imp. Univ. Tokyo, **7,** 353.
Maximov, A. A., and Bloom, W., 1946. *Text-book of Histology,* 4th ed. Phil. and London (W. B. Saunders).
Nathanson, I. T., and Brues, A. M., 1941. Endocrinology, **29,** 397.
Parkes, A. S., 1945. Phys. Rev., **25,** 203.
Polak, M., 1946. Rev. Asoc. Med. Argent., **60,** 9.
Salmon, T. N., and Zwemer, R. L., 1941. Anat. Rec., **80,** 421.

SARASON, E. L., 1943. Arch. Path., **35**, 373.
SCHMIDT, I. G., and SCHMIDT, L. H., 1938. Endocrinology, **23**, 559.
SWANN, H. G., 1940. Phys. Rev., **20**, 493.
TAKEWAKI, K., 1936. J. Facult. Sci., Tokyo, **4**, 277.
TAMURA, Y., 1926. J. exp. Biol., **4**, 81.
TONUTTI, E., 1941.[1] Z. f. mik. anat. Forsch., **50**, 283.
—— 1942*a*.[1] Z. f. mik. anat. Forsch., **51**, 346.
—— 1942*b*.[1] Z. f. mik. anat. Forsch., **52**, 32.
TURNER, C. D., 1939. Anat. Rec., **73**, 145.
VACCAREZZA, A. J., 1946. Rev. Asoc. Med. Argent., **60**, 9.
WARING, H., 1935. Quart. J. micr. Sci., **79**, 329.
—— 1942. J. Endocrinol., **3**, 123.
—— and SCOTT, E., 1937. J. Anat., Lond., **71**, 299.
WHITEHEAD, R., 1933*a*. J. Anat., Lond., **67**, 387.
—— 1933*b*. J. Anat., Lond., **67**, 393.
—— 1933*c*. J. Anat., Lond., **67**, 399.
—— 1943. J. Path. and Bact., **55**, 392.
WOTTON, R. M., and ZWEMER, R. L., 1943. Anat. Rec., **86**, 409.
ZWEMER, R. L., 1936. Amer. J. Path., **12**, 107.
—— WOTTON, R. M., and NORKUS, M. G., 1938. Anat. Rec., **72**, 249.

[1] Quoted from Gruenwald (1946).

EXPLANATION OF PLATES

PLATE I

Fig. 1. A median longitudinal section of the right adrenal of an adult non-cancerous group male, aged 178 days. No zona reticularis evident. Bouin, Heidenhain's haem., Masson. ×40.

Fig. 2. A transverse section of the left adrenal of an adult cancerous group male, aged 186 days. No zona reticularis evident, the darker staining tissue around the medulla is the inner region of the zona fasciculata. This example has no brown degeneration and therefore is representative also of the adult non-cancerous male. Bouin, Ehrlich's haem. and eosin. ×40.

Fig. 3. A median longitudinal section of the right adrenal of a parous non-cancerous group female, aged 272 days. The deeply staining, irregular zona reticularis surrounding the medulla can be seen. Compare this gland with that of the male in Fig. 1. Bouin, Ehrlich's haem. and eosin. ×40.

Fig. 4. A transverse section of the left adrenal of a parous cancerous female, aged 146 days. The columns of the zona fasciculata cells are visible; they merge into the deeply staining zona reticularis which is clear but not extensive. This example has no brown degeneration and is therefore representative also of many non-cancerous group females. Bouin, Heidenhain's haem., and Masson. ×40.

Fig. 5. A longitudinal section (not quite median) of the right adrenal of a cancerous group parous female, CB sub-strain, aged 286 days, showing an advanced condition of brown degeneration (Grade 6). The lobules can be seen at the cortex-medulla border. No zona reticularis, as shown in Fig. 3, can be seen. Bouin, Heidenhain's haem., and Masson. ×40.

PLATE II

Fig. 6. A portion of the adrenal of a non-cancerous parous female, aged 272 days, shown in Fig. 3. The cells of the inner region of the zona fasciculata merge into the irregular zona reticularis. The medullary capsule can be seen, and, below, the medulla. ×350.

Fig. 7. An extensive and vascular zona reticularis from a CBB parous female, aged 240 days. The gland shows no brown degeneration and the zona reticularis is characteristic also of some non-cancerous females. Bouin, Ehrlich's haem., and eosin. ×350.

Fig. 8. A part of the zona reticularis of a non-cancerous female, aged 403 days; it is a mass of senescent cells; the medullary capsule is very prominent. Bouin, Ehrlich's haem. and eosin, Mallory. ×350.

Fig. 9. Part of an adrenal of a non-cancerous parous female, aged 177 days, which shows no zona reticularis; the cells of the zona fasciculata abut on to the medullary capsule. Bouin, Ehrlich's haem., and Masson. ×350.

Fig. 10. A sector of the adrenal of a non-cancerous group parous female, aged 163 days, showing the 'lipoid' free zone between the zona fasciculata and the medulla. Five per cent. formalin. Sudan III. ×40.

Fig. 11. A sector of the adrenal of a non-cancerous group parous female, aged 359 days, showing small lobules of 'lipoid' material (small brown degeneration lobules) in the zona reticularis. Five per cent. formalin. Sudan III. ×40.

Plate III

Fig. 12. The innermost cortical layer of the adrenal shown in Fig. 1. No zona reticularis, the fuchsinophil cells of the inner zona fasciculata abut on to the medullary capsule which is thin. ×350.

Fig. 13. The zona reticularis of a non-cancerous group male, aged 152 days. It has the characteristics of the normal female zona reticularis but it is not so extensive. Bouin, Ehrlich's haem. and eosin. ×350.

Fig. 14. Part of the gland shown in Fig. 5. The top of the photograph shows cells of the inner zona fasciculata with stages in the formation of brown degeneration; lower, the vacuolated lobules with pycnotic nuclei typical of brown degeneration. Part of the medullary capsule is to be seen at the bottom of the photograph and then the medulla itself. ×350.

Fig. 15. Part of the right adrenal of a CB parous female, aged 310 days. Brown degeneration lobules are at the periphery of the medulla, bounded, on the cortical side, by the medullary capsule. The latter is interrupted centrally. The lobules consist of degenerated cortical cells. Bouin, Heidenhain's haem., Masson. ×350.

Fig. 16. A sector of the left adrenal of a CB parous female, aged 356 days. The lobules of brown degeneration are to be seen between the cortex and the medulla. Five per cent. formalin. Sudan III. ×40.

Fig. 17. A small portion of a large lobule of brown degeneration chosen to show the cortical nature of some of the nuclei which have not completely degenerated and the remains of the cell wall around one of them (bottom right). Bouin, Ehrlich's haem. and eosin. ×700.

Plate IV (all figures ×700)

Figs. 18–25. Stages in the formation of a lobule of brown degeneration. (From Bouin, Ehrlich's or Heidenhain's haem. and eosin preps.)

Fig. 18. A cell of the inner region of the zona fasciculata showing vacuolization.

Fig. 19. A similar cell with the vacuolization increased.

Fig. 20. A cell from the same region, the cytoplasm completely vacuolated, the nucleus showing pycnosis.

Fig. 21. Two cells of the inner region of the zona fasciculata which have merged. The cell walls are barely discernible, the cytoplasm vacuolated, the nuclei showing pycnosis.

Fig. 22. A small lobule of brown degeneration formed by the coalescence of five cells. The nuclei have not completely degenerated and are of the cortical type. The lobule is situated in the last row of zona fasciculata cells bordering on to the zona reticularis.

Fig. 23. A small lobule of brown degeneration lying against the medullary capsule and surrounded by the blood spaces and collapsed cells typical of a normal zona reticularis.

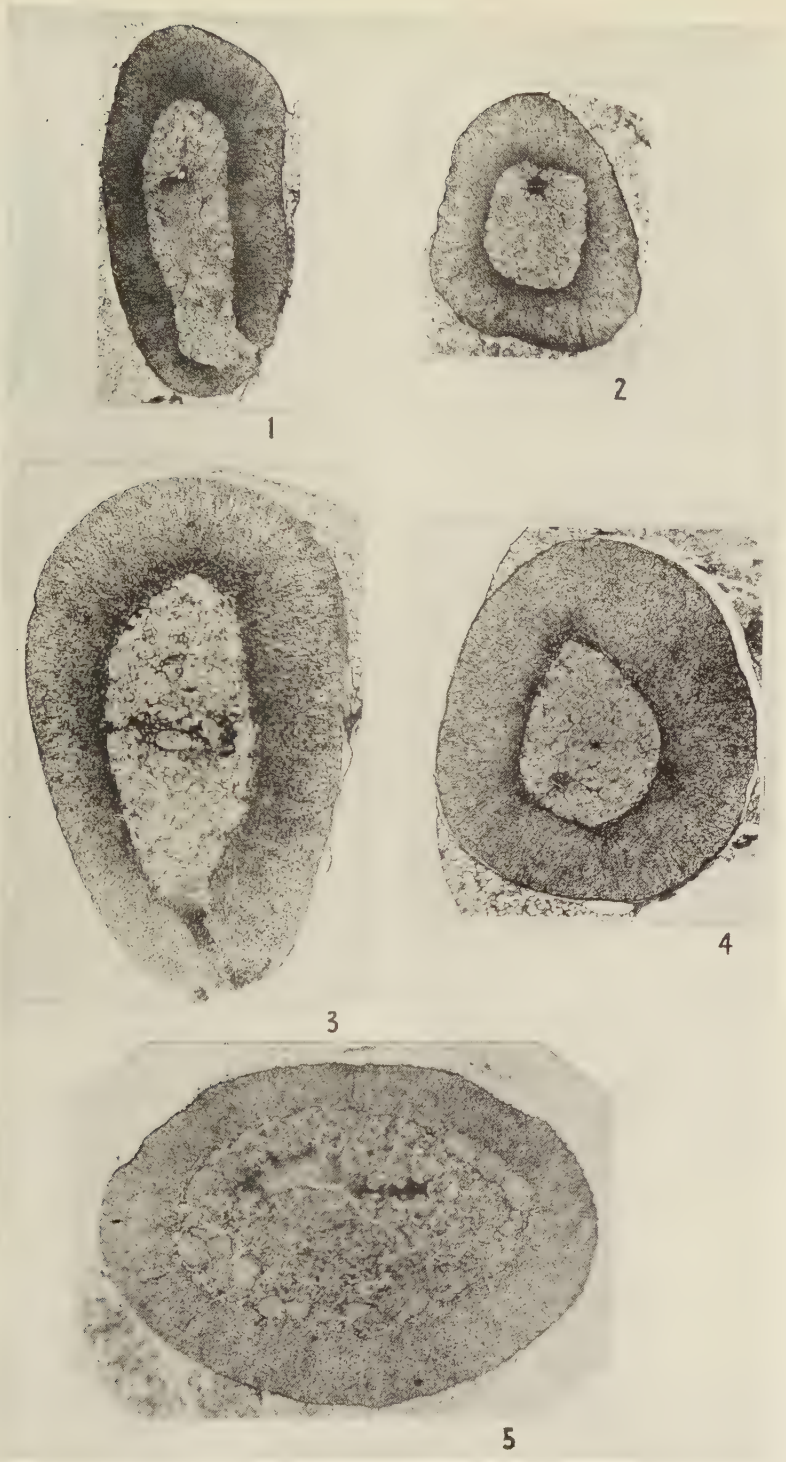
Fig. 24. Two small lobules of brown degeneration coalescing to form a larger.

Fig. 25. A larger lobule of brown degeneration lying against the medullary capsule. The degenerating nuclei are of the cortical type, the cytoplasm brown and vacuolated.

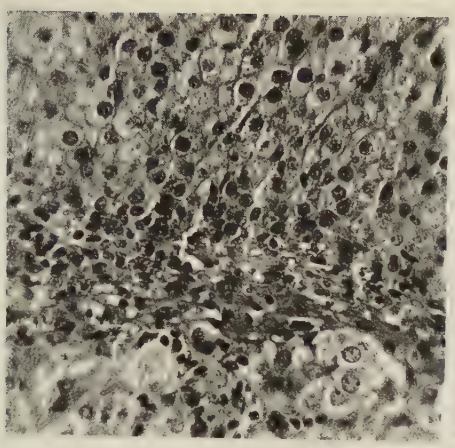
Fig. 26. Medullary cells showing brown degeneration. The reticulum of the medulla can be made out. Note the larger size of the medullary nuclei, compared with the cortical in Figs. 18–25.

Fig. 27. A usual position for a mitotic figure in the zona glomerulosa. Also note the narrow, clearly defined, adrenal capsule. Bouin, Ehrlich's haem. and eosin.

Fig. 28. A cell of the zona glomerulosa, dividing. It is against the adrenal capsule. Bouin, Ehrlich's haem. and eosin.



I. CHESTER JONES.—PLATE I



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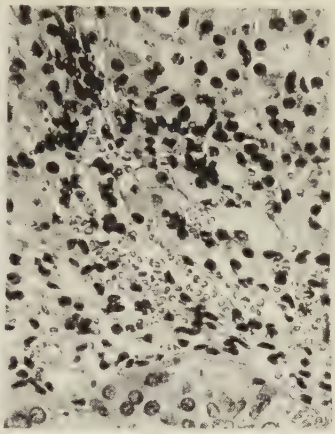

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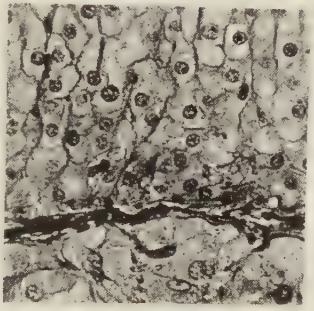

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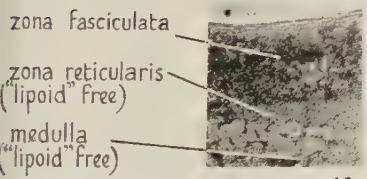

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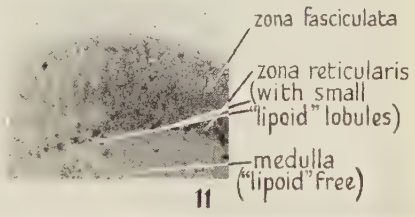

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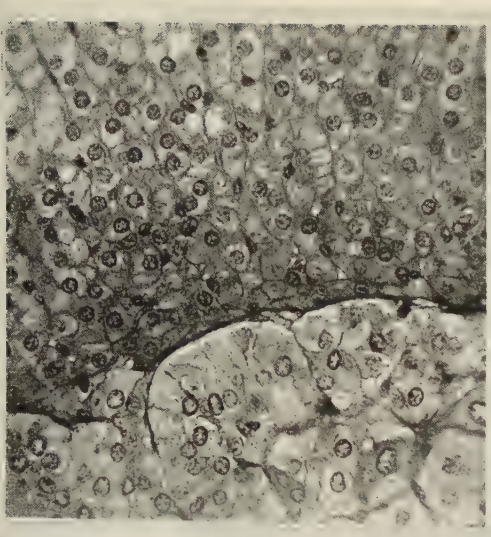

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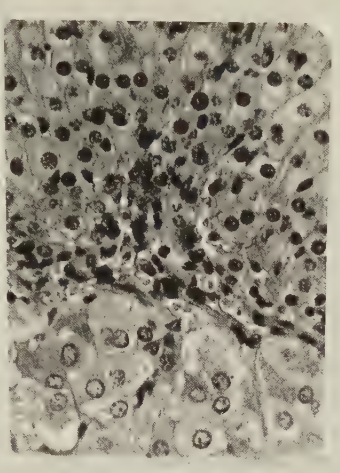

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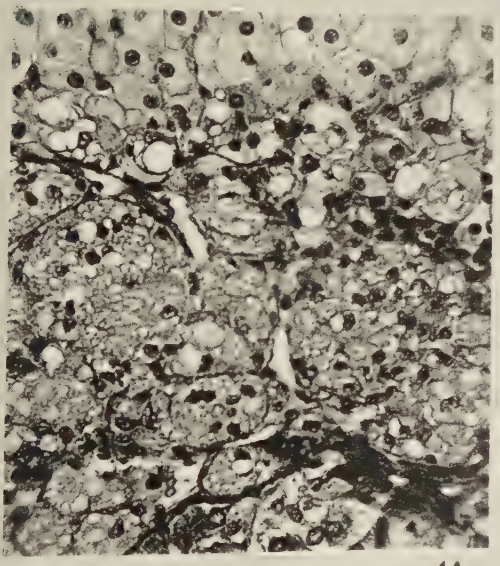

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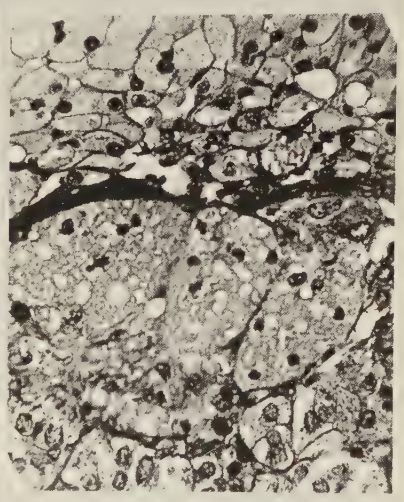

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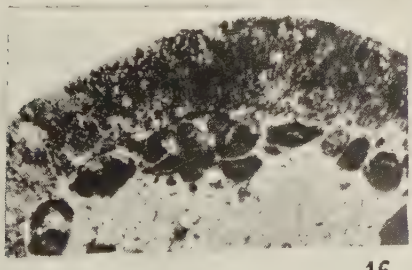

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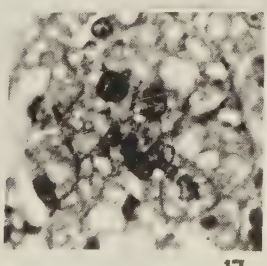

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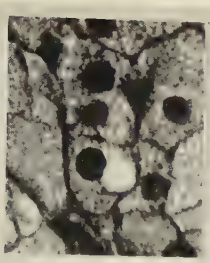
I. CHESTER JONES.—PLATE III



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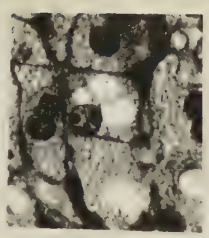

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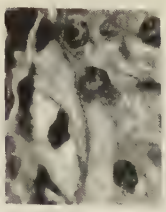

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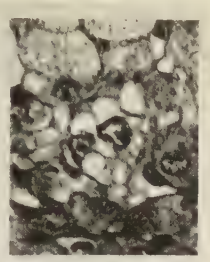

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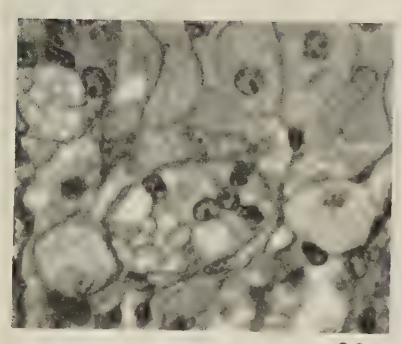

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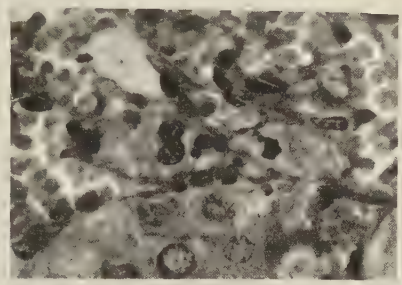

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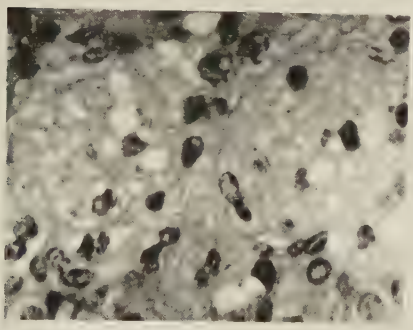

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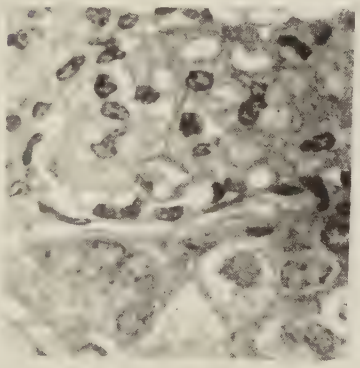

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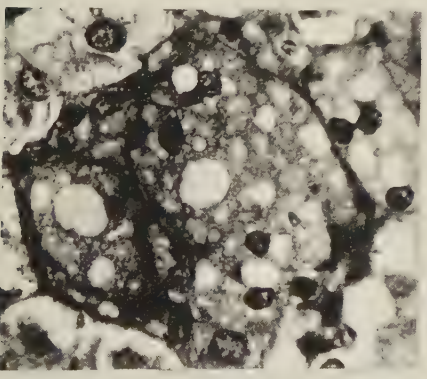

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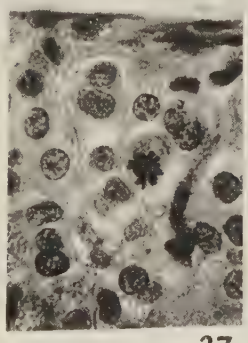

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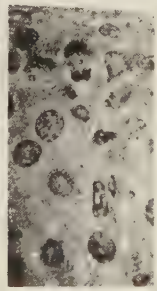

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I. CHESTER JONES.—PLATE IV

Cytology of the Glands associated with the Alimentary Tract of Domestic Fowl (*Gallus domesticus*)

BY

K. S. CHODNIK

(*From the Department of Zoology, University of Edinburgh*)

With twenty-nine Text-figures

Introduction

In a previous contribution the writer described the cytoplasmic components in the cells of the alimentary tract of the domestic fowl during different phases of cell activity (Chodnik, 1947). The present account records similar observations on the salivary glands, pancreas, and liver. So far as the writer is aware there are no accounts of the cytology of these glands in birds other than those on the chick liver (Dalton, 1933; Kater, 1933 and 1937). There are, however, many papers on the cytology of the glands associated with the alimentary tract of other animals.

Material and Methods

The material was obtained from birds which were used for the study on the alimentary tract; the same methods of fixation and subsequent treatment were employed. Material was fixed after a 24-hours' fast, and at periods from half an hour to 6 hours after a meal. The liver appears to be the most difficult tissue, as regards both penetration and fixation, especially with fluids containing osmium tetroxide. Regaud's technique proved to be the best for mitochondria. In the pancreas, however, it gave as a rule an uneven fixation of the superficial and deeper parts of the tissue; in the case of this gland Meves's method was preferable. For the study of the secretory process of the salivary glands the useful material was that treated according to Regaud's technique and counter-stained with Southgate's mucicarmine. To obtain a successful preparation of the Golgi material in the liver, it is essential to reduce the osmium tetroxide (after Mann-Kopsch, or preferably after Ludford's modification) in distilled water for at least 2 days at 35–7° C.

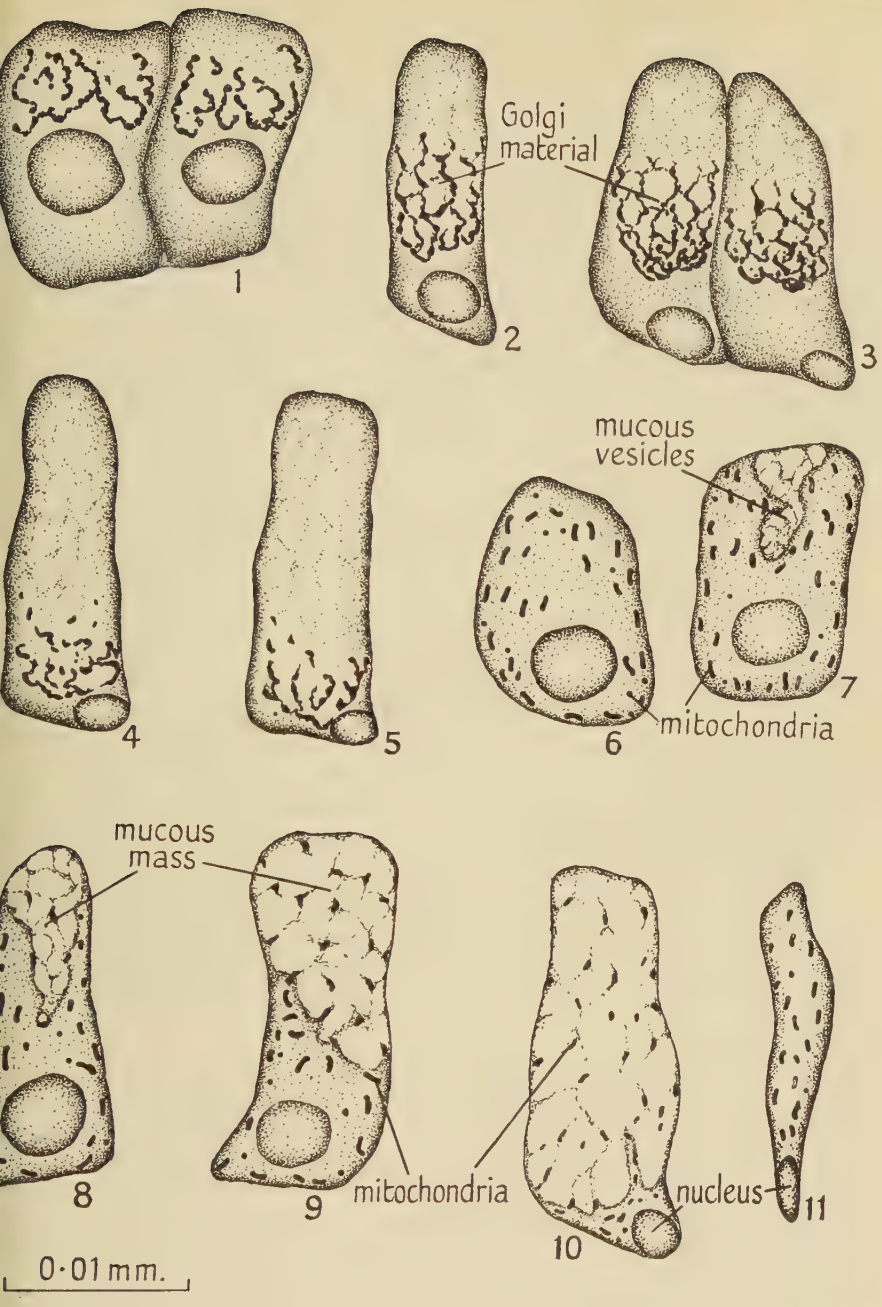
Observations

Salivary Glands

In the preliminary work single anatomical agglomerations of the glands in the mouth cavity, and samples of glands scattered on the dorsal part of the tongue and throughout the entire length of the oesophagus, were dealt with separately. Owing to their similarity all these glands were dealt with in the subsequent work as one unit. As regards the general features of the salivary glands of the domestic fowl it appears that, in contrast to the variety of cells

encountered in the salivary glands of mammals, there is great simplicity uniformity in avian material, and that the nature of the sceretory materi exclusively mucous (Heindrich, 1907; Calhoun, 1933). The epithelium the salivary glands consists of a single layer of cylindrical cells. Ther considerable variation in the size and shape of the cells depending u their position and the phase of secretory activity. Mitotic figures were observed.

The most common type of cell present, after a 24-hours' fast and at any t after feeding, is an elongated cylindrical form with very faintly mar borders. These cells are completely filled with accumulated secre material which gives a characteristic reaction with the dyes used to dem strate mucus (mucicarmine, toluidine blue, thionine). A small spher nucleus lies close to the basal membrane. The accumulated secretory mate is in the form of vesicles, probably of a semifluid consistency, which divided from each other by narrow cytoplasmic strips joined together forming a reticulated structure (Text-figs. 8–10). Mitochondria and G material are distributed inside the cytoplasmic strips. Feeding induces v little change in the majority of cells, but after a meal a certain number, wh is usually very limited, discharge their content into the glandular lur where it may be easily demonstrated. Due to the limited number of s cells, very careful examination of the sections is necessary in order to fol the consecutive phases of secretion. It was observed that when a cel stimulated to discharge its contents the process of evacuation, which co mences with the rupture of the cell membrane next to the lumen, contir until the cell is completely empty. Following the evacuation of the secret the cell becomes narrow and small, and before it enters upon a new phas activity, passes through a transitory regenerative phase. It enlarges gradu and refills with cytoplasm. The nucleus moves slowly from the basal towards the middle region of the cell. Having accomplished its regenera and the reorganization of all the cytoplasmic components the cell is re again to enter upon a period of secretion. In the early phase of secre small mucous vesicles appear in the cytoplasm in the region between nucleus and the lumen (Text-fig. 7). The vesicles increase in number size and at the same time move towards the glandular pole of the cell, wh a vesicular area of secretory material is formed (Text-figs. 8 and 9). T area expands gradually towards the basal part of the cell displacing mos the cytoplasm. The remaining cytoplasm forms narrow strips which di and separate the mucous mass into large vesicles. During the accumula of the secretory material the nucleus moves towards the basal part of the The descent of the nucleus, the non-disappearance of the cytoplasmic c ponents, and the general lack of mitotic figures, suggest that the cell d not disintegrate but regenerates after a secretory cycle and again beco active. Narrow cells seen among the young cells are probably in the ear stages of regeneration (Text-fig. 11). The early secretory phases are descri in the section dealing with the Golgi material.



TEXT-FIGS. 1–11. All figures from salivary glands.

FIGS. 1–5 from Kolatchev preparations, showing Golgi material.

FIGS. 6–11 from Champy-Kull and Regaud preparations, showing mitochondria.

Fig. 1.—Regenerated cells before entering upon secretion, Golgi material hypertrophied. Fig. 2.—l with secretory granules along the Golgi threads, mucous vesicles surrounded by the ramifications the Golgi material. Figs. 3 and 4.—Cells in advanced stages of secretion. Fig. 5.—Cell in resting ge, totally filled with mucous secretion, Golgi material reduced in amount. Fig. 6.—Regenerated , showing mitochondria. Fig. 7.—Cell in early stages of secretion; mucous vesicles are formed. s. 8 and 9.—Cells in advanced stages of secretion, mucous mass expands. Fig. 10.—Cells in resting ge, totally filled with secretion. Fig. 11.—Cell after extrusion of secretory material.

Golgi material

The reduced osmium in the Golgi region is in the form of a network. undergoes marked morphological changes correlated with the different phas of secretory activity. In a dormant cell with the maximum accumulation secretion, the Golgi material is often difficult to impregnate, and consists o few threads with small thickenings. It lies quite close to the basal membra and to the nucleus. Some of the threads follow the cytoplasmic partitio between the secretory vesicles (Text-figs. 4 and 5). Parallel with the regener tion of the cell the Golgi material increases in amount and shows a distin tendency to spread over a large area always above the nucleus (Text-figs. and 3). In the fully regenerated cell the Golgi material is a strongly hypertr phied structure consisting of lamellar spirals, branched threads, and vario short rods and granules (Text-figs. 1 and 2). With the accumulation of t secretory product the Golgi material is gradually pushed towards the bas part of the cell. At the same time its size and power to reduce osmiu tetroxide diminish. Finally, it returns to the barely perceptible form wi which this description started.

Numerous small granules are present along the faintly outlined threads the hypertrophied Golgi material of the regenerated cell, particularly in ce prepared by the Mann-Kopsch technique. These are, no doubt, prima secretory granules which later increase slightly in size. Some of the larg granules assume a vesicular form and their power to reduce osmium tetroxi decreases. Later the vesicles no longer reduce osmium tetroxide and no possess an affinity for the dyes used for the demonstration of mucus. U fortunately it was not possible to follow all the stages of the transformati of the primary secretory granules into mucous vesicles. The larger secreto vesicles are surrounded by the ramifications of the Golgi material whi separate them into clusters (Text-figs. 2 and 3). With the accumulation more material the vesicles tend to fuse and disrupt the outer links of the Go material, which moves towards the basal and lateral cell boundaries.

Mitochondria

The mitochondria of regenerated cells which have not yet become acti are present as numerous rods, long filaments, and granules. Granules a most numerous in the basal subnuclear part of the cell, while rods and fil ments predominate in other parts of the cytoplasm and are more or less even distributed. Both rods and filaments are arranged parallel to the long ax of the cell (Text-fig. 6). In older cells, under the pressure of the secreto material, the majority of the mitochondria retreat to the cytoplasm in the bas and peripheral regions; a few, however, are scattered in the cytoplasm betwe the vesicular areas (Text-figs. 9 and 10). In the cells totally filled with secr tion, granules and short rods are the prevalent forms. It is difficult, howeve to determine if there is an actual decrease in the number of mitochondr present during the dormant phase as many small forms situated between t mucous masses may easily be overlooked. Although a few mitochondria a

ually scattered on the periphery of the mass of secretion, there is no evidence at they give rise to secretory material.

Pancreas

The following account refers to the acinar cells; the cells of the islets of ngerhans were not studied. In spite of the ease with which good cyto-gical preparations were obtained, and the large size of the cells, the acinar lls are difficult to study when food is used to stimulate the secretory pro-sses. The cells composing an acinus are of an irregular pyramidal shape. large spherical nucleus lies near the basal membrane or close to the middle gion of the cell depending upon the functional stage. In the normal resting ase, subsequent to a 24-hours' fast, the spherical secretory (zymogenic) anules totally fill the portion of the cell between the nucleus and the lumen the acinus. They vary considerably in size and staining properties. The naller granules usually stain deeply with acid fuchsin, while the larger ones ain a very faint pink or lose their affinity for acid fuchsin and take on a llow colour from the picric acid used in differentiation. Granules very rely extend to the extreme basal part of the cell. Soon after feeding (half an our) a large number of secretory granules are evacuated, but total evacuation as never observed. In many cells, unstained vacuoles mark the position rmerly occupied by secretory granules.

The present observation confirms previous statements (Hirsch, 1932; Ries, 935) that the pancreatic acinus acts as an autonomous unit. Some of the ini, fixed half an hour after feeding, contain very few granules, while others ay contain a large number and remain in an unchanged condition similar the resting phase. It was not possible to follow the order in which the acuation of the granules takes place. The release of the granules, which egins soon after feeding, continues for about 2 hours after the intake of food. appears that as soon as the discharge of secretion begins, the formation of ew granules takes place. Numerous small granules become visible in the upranuclear zone. These early secretory granules stain very deeply with acid chsin, in contrast to the older granules, which take on a faint stain. Granules ere observed in fixed material, in material stained supravitally with neutral d, and in unstained cells in which they appear as highly refractile bodies. wo hours after feeding, there is great variation between the different acini; me are entering upon the secretory phase while others contain large secre-ry granules situated between numerous small ones. The process of restitution rogresses gradually to the stage observed after a 24-hours' fast, and terminates out 5–6 hours after feeding. In spite of a careful search of fixed and stained reparations granules were not observed in the subnuclear zone, but small anules were visible in this region of cells which were stained supravitally.

olgi material

The Golgi material was identified in acinar cells during a fast, when there a maximal accumulation of secretory granules, and at each stage subsequent

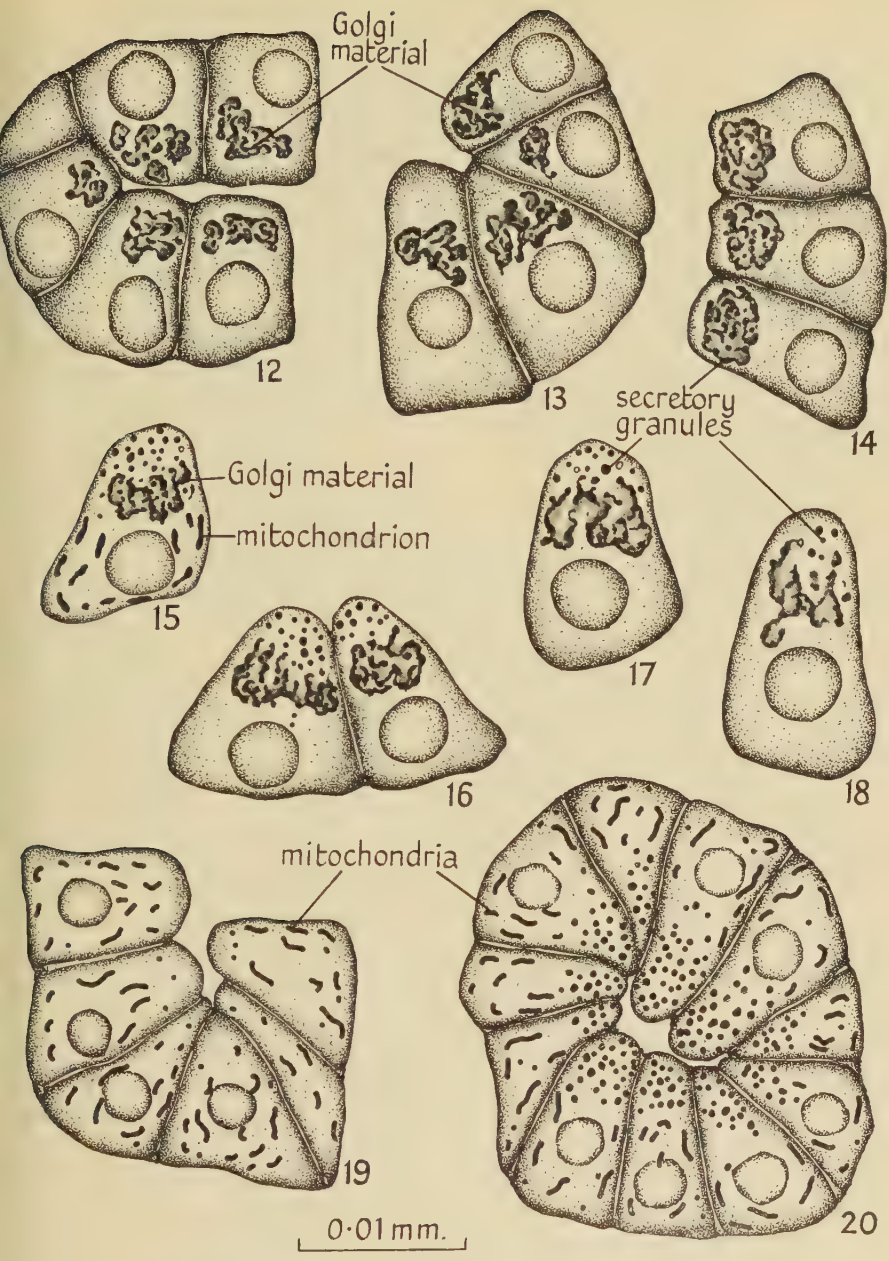
to feeding. It appears to form a support and framework for a substance which reduces osmium tetroxide slightly more vigorously than the rest of the cytoplasm (Text-fig. 12). Marked changes were noted in the Golgi material during the production of secretion. After a 24-hours' fast the Golgi material forms a compact, sharply outlined body which lies in the supranuclear region surrounded by the secretory granules. The first visible morphological changes are evident 1 hour after feeding, when there is a loosening of the osmiophilic threads and the field covered by the Golgi material is considerably larger than during a fast. Small sharply outlined swellings are present on the osmiophilic threads. The substance which fills the spaces between the osmiophilic framework reduces osmium tetroxide more vigorously than during the inactive phase (Text-fig. 13); later, there is a further increase in its reducing power. Two hours after feeding the osmiophilic threads become less clearly outlined and numerous granules are now visible along their course (Text-fig. 14). In the next phase, about 3 hours after feeding, the osmiophilic threads on the glandular side of the Golgi material appear to lose their continuity and secretory granules possessing a narrow osmiophilic rim move away from the Golgi field (Text-fig. 16), and proceed towards the extreme glandular pole of the cell. In about 5–6 hours after feeding the Golgi material almost regains the form and properties characteristic of the resting stage.

Mitochondria

The mitochondria of the acinar cells of the domestic fowl are of a similar pattern to that described by most workers on the pancreas of other animals. They are remarkable for their size and length. A great variety of forms are encountered in each cell—long filaments, often extending for two-thirds the length of the cell, short rods, and granules. The filaments are generally slightly tortuous. All the mitochondria appear to lie for the most part parallel to the long axis of the cell, but some may lie parallel to the basal membrane (Text-figs. 19 and 20). In the osmium fixed material the presence of secretory granules renders the examination of the mitochondria in the supranuclear zone difficult, and often impossible (resting stage). In material fixed and stained according to Regaud's technique, the mitochondria are clearly visible in all parts of the cell (Text-fig. 19). The long filaments are commonly seen along the lateral cell walls. At the time of increased cellular activity, long tortuous forms are very seldom seen and more regular rods prevail. This is most notable 2–3 hours after feeding.

Liver

Dalton (1933) gave a thorough description of the changes in the cytoplasmic components of the cells of the liver of the chicken from the earliest embryonic stages to the time of hatching and also a short account of the changes caused by fasting and feeding. He stated that, although the mitochondria and Golgi material assume characteristic patterns correlated with increased and decreased cellular activity, there is no evidence of a morphological character which



TEXT-FIGS. 12–20. All figures from pancreas.

FIGS. 12–18 from Kolatchev preparations, showing Golgi material.

FIG. 19 from Regaud preparations, showing mitochondria.

FIG. 20 from Meves preparations, showing mitochondria and secretory granules.

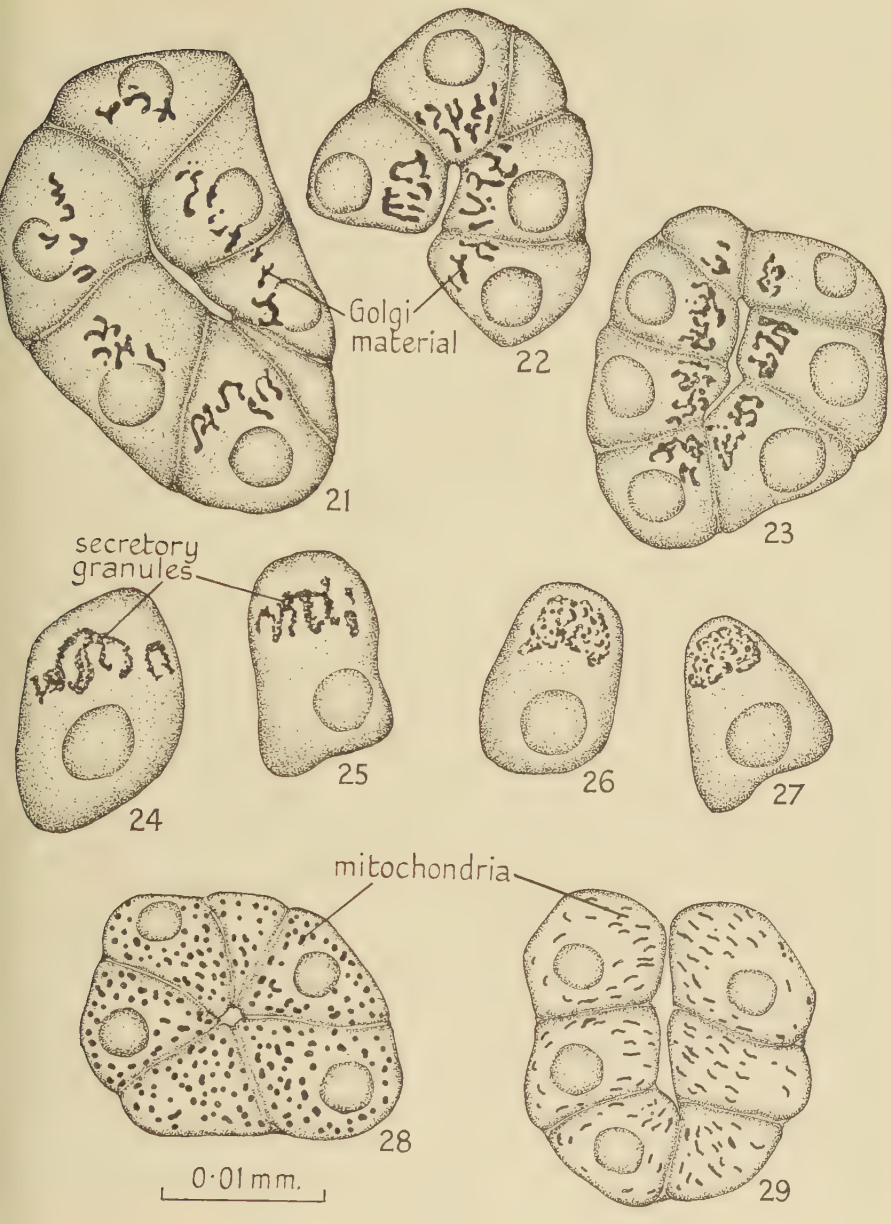
ig. 12.—Cells after a 24-hours' fast. Fig. 13.—Cells 1 hour after feeding. Fig. 14.—Cells 2 hours r feeding, formation of secretory granules. Fig. 15.—Cell 2 hours after feeding, showing Golgi erial, mitochondria, and secretory granules. Fig. 16.—Cells 3 hours after feeding, secretory nules accumulate on the glandular pole. Figs. 17 and 18.—Cells from birds with constant access food, pictures as in advanced stages of secretion. Fig. 19.—Cells showing mitochondria. Fig. —Cells showing mitochondria and secretory granules.

would indicate a specific action of the mitochondria, or the Golgi material in secretion. During the present investigation, which is an extension of Dalton's work, some interesting results were obtained.

The liver of the domestic fowl is much simpler than that of mammals, and has no true lobular structure. The liver epithelium surrounds the tubules which build the intercellular bile canaliculi. The hepatic cell is an irregular polyhedral pyramid with its apex bordering on the lumen of the bile canaliculus and the basal part in contact with the blood capillaries. The large spherical nucleus lies in the basal part of the cell, and a few globules of fat are usually seen in the surrounding cytoplasm. A thorough search did not reveal any interlobular variations whatsoever. In this paper no reference is made to the glycogen–glucose equilibrium or to the secretion of bile.

Golgi material

Until the works of Cramer and Ludford (1927), who successfully impregnated the Golgi material of certain mammals, nothing was known about this component of the liver cell. During the present work a comparison of material fixed after a 24-hours' fast with preparations from birds killed at hourly intervals after feeding showed that the form of the Golgi material is very closely correlated with the phases of secretion. In the cells of fasting chickens the Golgi material is located comparatively close to the nucleus. It consists of somewhat thick sausage-like rods, with a few threads between them. These rods are of fairly uniform thickness and are loosely placed in the form of an umbrella above the nucleus (Text-fig. 21). The first changes in the Golgi material were perceptible 1 hour after the intake of food. The thick Golgi rods elongate slightly, become thinner, and arrange themselves above the nucleus like the rays of an open umbrella. At this phase the connecting threads are no longer present (Text-fig. 22). Two hours after a meal, the long rods become more twisted and are again connected with one another by threads, thus forming a considerably more complicated structure. Thickenings and a few granules are visible along the Golgi threads; these react to osmium tetroxide in the same manner as the threads themselves (Text-fig. 23). Four hours after feeding, the Golgi material expands considerably and numerous granules, arranged in rows, replace many of the links. It often appears as though the threads are split into thinner threads with a large number of granules intimately connected with them. At this time most of the granules are small and deeply blackened while the connecting threads are much fainter in outline (Text-figs. 24 and 25). Six hours after feeding, some granules are situated beyond the Golgi field in the form of large vesicles which are very faintly outlined in osmic preparations. The thick threads are replaced by grey lamellae which spread between the small dark granules. The substance between the Golgi links and the granules reduces osmium tetroxide strongly and takes on a darker colour, thus outlining the whole Golgi field (Text-figs. 26 and 27). In the hepatic cells of birds which have constant access to food the Golgi rods are thicker and more deeply impregnated, but are similar in



TEXT-FIGS. 21–9. All figures from liver.

FIGS. 21–7 from Ludford preparations, showing Golgi material.

FIGS. 28–9 from Regaud preparations, showing mitochondria.

Fig. 21.—Cells after a 24-hours' fast. Fig. 22.—Cells 1 hour after feeding. Fig. 23.—Cells 2 hours after feeding, enlargement of the Golgi material. Figs. 24 and 25.—Cells 4 hours after feeding, secretory granules seen along the Golgi links. Figs. 26 and 27.—Cells 6 hours after feeding, strong hypertrophy of Golgi material, secretory granules in Golgi field. Fig. 28.—Cells after a 24-hours' fast showing mitochondria. Fig. 29.—Cells 2 hours after feeding, showing mitochondria arranged parallel to the long axis of the cells.

shape to those of the resting stage. In this condition a few granules between and along the thick threads and rods may generally be observed. The whole secretory cycle in the liver cell extends over a period of 6 hours after the intake of food. This is the longest cycle observed in the tissues of the fowl and renders the liver a useful organ in which to follow the consecutive phases. The first secretory granules appear in intimate association with the Golgi material. In the later stages their number increases considerably, but they continue to remain for some time closely connected with the Golgi material and are linked together into an organized body by the osmiophilic lamellae (Text-figs. 26 and 27). The subsequent freeing of the granules from the Golgi field is analogous to the process which takes place in the pancreas.

Mitochondria

The mitochondria of the hepatic cells show more striking changes during increased cellular activity than in any of the other gland cells investigated by the writer. After a 24-hours' fast, the mitochondria are in the form of granules, ovoid bodies, and short rods. Granules and ovoid forms are most numerous and are present almost exclusively in the basal part of the cell. Rods intermingled with granules are seen in the supranuclear zone. The supranuclear mitochondria tend to be arranged more or less parallel to the long axis of the cell, while those in the subnuclear region are scattered at random (Text-fig. 28). A striking change in the morphological pattern commences 1 hour after feeding and is very prominent after 2 hours, when only a few granules are present while rods and filaments are the predominant forms. The mitochondria appear to be more numerous, much thinner, and slightly wavy; they have a typical polar orientation from the basal to the glandular pole, and are more or less equally distributed throughout the cytoplasm (Text-fig. 29). From 2 hours after feeding no further change in the mitochondria takes place, but in many cells light irregular areas free of mitochondria appear round the nucleus. The mitochondria are pushed aside and accumulate on the border of these irregular areas, which give a fenestrated appearance to the cytoplasm around the nucleus. These areas correspond closely with those occupied by glycogen which accumulates some hours after feeding. In the material taken 4–5 hours after feeding and fixed by Meves's technique numerous small granules were seen near the glandular pole of the cell and in the Golgi field. The mitochondrial picture of birds with constant access to food does not show any marked difference from that observed in the later hours after feeding. Filaments are predominant, but rods appear to be more numerous than in the early phases of digestion.

Discussion

Salivary glands

The secretory cycle in the salivary glands of birds resembles in many respects that of the salivary glands of *Tipula paludosa* (Gresson, 1937). There appears to be a similarity in the formation and evacuation of the secretion

nd in the regeneration of the cell after each secretory cycle. Unlike the work n the salivary gland cells of insects the present investigation shows a close elationship between the secretory process and the changes undergone by the olgi material. In that respect the present material appears to be more uitable for determining the relationship between the cytoplasmic components nd the secretory product. The strong hypertrophy of the Golgi material in ne early phases of secretion, and the presence of numerous granules, which nlarge and become vesicular while in close contact with the Golgi material, re undoubtedly associated with the origin of the secretion of the salivary glands f the fowl. This agrees with previous observations on mucus-secreting cells Florey, 1932; Duthie, 1933) and is similar to the secretory process observed n intestinal goblet cells (Florey, 1932). Although the mitochondria do not ppear to show such a close association with the formation of secretion, as ecorded in other gland cells, nevertheless, their arrangement and constant resence on the surface of the mucous vesicles intermingled with the Golgi naterial indicates that they participate in the process.

ancreas

The acinar cells of the pancreas have been more extensively studied than ne cells of any other vertebrate gland. The majority of workers used power- ul stimulants (such as pilocarpine) to evacuate the secretion. When food is sed as a stimulant to bring about the discharge of the secretion, as it was uring the present work, a comparatively small number of secretory granules evacuated and observation is therefore rendered much more difficult. In pite of these difficulties the consecutive phases of the secretory cycle, ac- ompanied by morphological changes of the cytoplasmic components, were llowed with considerable clarity. The behaviour of the Golgi material rongly suggests that it plays an important part in secretory activity.

The present account on the secretory cycle in the acinar cells agrees with arlier descriptions (beginning with Nassonov's paper, 1923) which drew ttention to the connexion between the Golgi material and the origin of the ranules of secretion. Unfortunately, since fixed material was chiefly used, was not possible to identify the secretory granules prior to their appearance n the Golgi field. It was therefore impossible to confirm the work of Covell 928), Hirsch (1932), and Duthie (1933) who stated that there is a con- exion between the mitochondria and the early phases of the origin of ecretory granules.

iver

The dual role played by the liver cells in the glycogen–glucose equilibrium nd the bile secretion and the characteristic lobular structure of the liver has imulated much research on the function of these cells under different hysiological conditions. In particular the function of the mitochondria in ne hepatic lobule has been the subject of much speculation. The function f the mitochondria has been ascribed either to carbohydrate assimilation

(McA. Kater, 1931, 1937; Clark and Hair, 1932), or to the secretion of bile (Cramer and Ludford, 1926–7). It appears to be generally accepted that the presence of spherical mitochondria indicates an inactive phase and that the filamentous type is present during secretory activity (Dalton, 1933; McCudry, 1939, 1940; Steffens, 1941). All the earlier workers mention the great technical difficulties involved in studying the hepatic cell, especially in demonstrating the Golgi material. It is almost certain that works based on silver nitrate impregnation must be treated very sceptically, because the most reliable observations have shown that silver nitrate fails to impregnate liver cells (Cramer and Ludford, 1926–7; Pollister, 1932; Dalton, 1933; Subramaniam, 1938). In spite of the difficulties encountered very spectacular morphological changes, both of the Golgi material and mitochondria, were observed during the present investigation. All changes observed in the cytoplasmic components of the liver cell are closely connected with cellular activity and secretory phenomena. The very slow morphological changes observed in the Golgi material render the hepatic cell of the fowl ideal material for the study of the phases of secretion. It is very evident that the granules of secretion appear in close topographical relationship to the Golgi material and that the Golgi material undergoes changes of form and disposition which are correlated step by step with the stages of secretory activity. The present observation on the Golgi material in the liver cell agrees very closely in nearly every respect with Subramaniam's studies on the Golgi material of the liver of *Rhacophorus maculatus* Gray (1938). The morphological alteration of the mitochondria is no less closely connected with the increase of cellular activity. One hour after feeding, the filamentous mitochondria become oriented parallel to the main axis of the cell; this change and the redisposition of mitochondria are much more striking than any which occur in the glandular epithelia of the alimentary tract and other associated glands. Whatever the function of the mitochondria may be, their rhythm depends entirely on the time of feeding, and their response appears to be more rapid than that observed in the liver cells of other vertebrate animals. There is no justification for the conclusion drawn by McA. Kater (1933 and 1937) that the mitochondria show no regularity in the liver of the domestic fowl as regards their morphological features, and that the mitochondrial pattern is so irregular that it can never be regarded as normal. Kater's conclusion can only be attributed to the harmful effect of the stimulants used, or even to the methods of feeding which he employed. The present observation agrees with the generally accepted opinion that granular mitochondria occur during the inactive stage while filaments indicate functional activity.

Summary

Salivary glands

The onset of secretion is marked by hypertrophy of the Golgi material. During the secretory phase the mitochondria lie parallel to the long axis of the cell. Secretory granules are first visible in close association with the Golgi

aterial. Feeding brings about the evacuation of the secretion. Evacuation f secretion is total; regeneration of the cell precedes the next secretory hase.

ancreas

The Golgi material enlarges during the onset of secretion. Few changes ere observed in the morphology of the mitochondria. Secretory granules re first visible in close association with the Golgi material. Later they move owards the acinar lumen. Feeding brings about evacuation of the secretion ranules, but total evacuation does not take place.

iver

The morphology of the Golgi material changes considerably during the roduction of the secretory granules. Granular mitochondria predominate fter a 24-hours' fast. Rods and filaments are prevalent after feeding. During he secretory phase the mitochondria lie parallel to the long axis of the cell. The secretory granules are first visible in association with the Golgi material. ree secretory granules are not visible until 6 hours after feeding.

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REFERENCES

ALHOUN, M. L., 1933. Iowa St. Coll. J. Sci., **7**, 261.
HODNIK, K. S., 1947. Quart. J. micr. Sci., **88**, 419.
LARK, J. A., and HAIR, G. E., 1932. Z. Zellforsch. mikr. Anat., **15**, 123.
OVELL, W. P., 1928. Anat. Rec., **40**, 213.
RAMER, W., and LUDFORD, R. J., 1926–7. J. Physiol., **62**, 74.
ALTON, A. J., 1933. Anat. Rec., **58**, 321.
UTHIE, E. S., 1933. Proc. Roy. Soc. B, **113**, 459.
— 1934. Ibid., **114**, 20.
LOREY, H., 1932. Brit. J. exp. Path., **13**, 349.
RESSON, R. A. R., 1937. Z. Zellforsch. mikr. Anat., **25**, 131.
ALL, E. M., and MACKAY, E. M., 1933. Amer. J. Path., **9**, 205.
EINDRICH, K., 1907–8. Morph. Jb., **37**, 10.
IRSCH, G. C., 1931. Biol. Rev., **6**, 88.
— 1932. Z. Zellforsch. mikr. Anat., **15**, 36, 290.
ICA. KATER, J., 1931. Anat. Rec., **49**, 277.
— 1933. Z. Zellforsch. mikr. Anat., **17**, 217.
— 1937. Ibid., **25**, 127.
ICCUDRY, M. B. D., 1939. J. Morph., **64**, 9.
— 1940. Biol. Bull., **79**, 252.
ASSONOV, D., 1923–4. Arch. mikr. Anat., **97**, 136; **100**, 433.
OLLISTER, A. W., 1932. Amer. J. Anat., **50**, 179.
IES, E., 1935. Z. Zellforsch. mikr. Anat., **22**, 523.
TEFFENS, H. W., 1941. Anat. Rec., **81**, 243.
UBRAMANIAM, M. K., 1938. Proc. Indian Acad. Sci. B, **7**, 80, 125.

Observations on the Chemical Composition of Myelin and the Smallest Size of Myelinated Nerve-fibres in the Central Nervous System

BY

A. BRODAL AND R. G. HARRISON

From the Department of Human Anatomy, Oxford. The first-named author is on leave from the Anatomical Institute, University of Oslo, with a Rockefeller Fellowship.)

With one Plate

INTRODUCTION

THE methods of polarization optics and X-ray diffraction (Schmitt and Bear, 1937, 1939) have shown that the myelin sheath consists of thin heets of protein, wrapped concentrically about the axon, with two bimole- ular layers of lipoids interspersed between adjacent protein layers: there is irefringence due preponderantly to lipoids in the case of larger fibres, graded lown to that in smallest fibres resulting primarily from proteins, the transi- ion from lipoid to protein predominance occurring at a diameter of about μ. (The nomenclature used in this paper is as follows: Lipoids, a generic erm for all substances extractable from tissues by fat solvents include, amongst thers, lipides (lipids) all of which contain at least one fatty acid. These nclude the triglycerides, esters of fatty acids with other alcohols (such as terols) and lipines, which are those lipides containing nitrogen and some- imes also phosphorus, i.e. the phospholipines and galactolipines.) There is, owever, little information in the literature concerning the histo-chemical onstitution of myelin. Bielschowsky (1935) has stated that myelin has a omplex structure, containing cholesterol, lecithin (a phospholipine), 'Prota- on', and 'Cerebrin'. He also asserts that lecithin is the substance coloured y Weigert's method, although he does not give his reasons for saying so. age (1937), in his monograph on the chemistry of the brain, states that brain ssue is very rich in phosphatide (i.e. phospholipines), containing about twice s much as liver and kidney and three times as much as heart-muscle, much f it being contained in myelin sheaths, but the cells themselves not lacking it. t is therefore not surprising that 'phosphatides compose a large part of the pids in brain'. Koch and Koch (1913) determined that the most active period f medullation in albino rats is during the tenth to twentieth days post- artum and that during this period phosphatide formation is the predominant eature.

The current usage of the terms 'myelinated' and 'unmyelinated' may tend convey the impression that the two designations denote two fundamentally

different kinds of nerve-fibres. However, it appears to have become generally believed, although as far as is known not yet proved, that the distinction is mainly one of a quantitative nature. Thus, from an anatomical point of view, Ranson, Droegemueller, Davenport, and Fisher (1935) state that 'anatomically no sharp line of demarcation can be drawn between myelinated and unmyelinated fibres'. The fact that fibres of the same category may be unmyelinated in smaller animals and myelinated in other, larger, animals (e.g. the fibres of the ventral and dorsal roots as shown by Duncan, 1932, 1934) strongly supports this contention. Kiss and Mihalik (1929) from anatomical studies conclude that even the smallest nerve-fibres of the spinal nerves and roots have a myelin sheath, although it appears that the evidence on which their statement rests is somewhat equivocal. The findings in electrophysiological studies of nerve-fibre conduction do not lend support to the assumption of fundamental differences between fibres which are myelinated and those which are commonly regarded as being unmyelinated (see e.g. Blair and Erlanger, 1933; Grundfest and Gasser, 1938). By workers on nerve conduction it is commonly stated that the fibres of lowest conduction velocity, the C fibres, are mainly, at least, unmyelinated fibres (see e.g. Zotterman, 1939), and the two terms are sometimes used synonymously.

It appears that the lowest limit of the thickness of myelinated fibres is mainly a question of the methods applied in their study. According to the literature the finest myelinated fibres have been observed in osmium tetroxide preparations, which allow the tracing of somewhat finer fibres than the Weigert technique and its modifications. Several authors describe myelinated nerve-fibres in the spinal roots and peripheral nerves in osmium preparations of a diameter of 1–2μ (see e.g. Table 1 in Duncan, 1934; Foley and Dubois, 1943; Rexed, 1944, and many other authors). Some have even observed fibres less than 1μ in thickness (e.g. Duncan, 1934, in the pigeon; Hillarp and Olivecrona, 1946, a small proportion in some cases in the cervical sympathetic trunk in the rat). Using polarization optics and X-ray diffraction methods, Schmitt and Bear (1937, 1939) have succeeded in demonstrating that fibres of diameters less than 2μ possess a lipoid investment, its thickness decreasing with the thickness of the axons. However, the proportion of protein to lipoid appears to increase gradually with the fineness of the fibre, and it appears that the finest fibres, less than 1μ, cannot be satisfactorily studied by this technique, according to Schmitt and Bear.

In view of the data reviewed above, it was considered worth while to study the application of the histochemical method for detection of lipine worked out by Baker (1946) to the central nervous system. This method, which is claimed to be specific for phospholipines (Baker, 1947), has been applied by Baker to several tissues and has been used by Harrison and Cain (1947) in the study of the adrenal cortex. The present study was undertaken with the following aims in view: first, to gain information concerning the chemical constitution of myelin, and secondly, to determine the possibility of tracing myelinated fibres thinner than those revealed by other methods.

Material and Methods

The material used for this investigation consisted of human and rats' brains. The human brain was obtained from an individual not known to have suffered from any neurological disorder.[1] The rats' brains were from adult male rats. In addition to the use of Baker's method, other sections were treated with sudan black (see Baker, 1944) or nile blue (see Cain, 1947 *a* and *b*). The acid haematein test of Baker (1946) is a modification of the Smith-Dietrich test, used in conjunction with a pyridine extraction control test. A phospholipine is recognized by a blue or blue-black colour seen after acid haematein but not after pyridine extraction. A blue colour after pyridine extraction is due most commonly to acidic proteins but may, of course, be due to lipines not completely extracted. These acidic proteins also stain after acid haematein. The thickness of the fibres was measured by means of an eye-piece micrometer with oil immersion.

Observations

A. *Acid Haematein-stained Material*

Rat Material

In sections of the central nervous system of rats stained by the acid haematein test the myelinated fibre tracts and the white matter of the cerebrum and cerebellum are seen with the naked eye to acquire a pure blue tint as in a Weigert-Pal preparation. The grey matter appears grey-blue. On microscopical examination this is observed to be due to the staining of numerous fibres within the grey matter. The intensity of the colour depends on the thickness of the section. Thin sections, 6–10μ, are best suited for the study of details and were used in this investigation. Baker (1946) states that sections 10μ or less in thickness must be used; while thicker sections might well introduce sources of error, thinner ones do not lose in specificity by slightly heavier differentiation. The findings made on examination of various parts of the central nervous system of the rat will be described below.

Cerebral Cortex. With the low power a fair number of coarser radiating fibres can be followed through all cortical layers into layer II. In addition some finer radiating and a certain number of tangential and oblique fibres are seen. When the sections are studied with higher power or with oil immersion, a multitude of finer and coarser blue streaks and minute blue spots and rings interspersed between the cells and coarser fibres are recognized (Pl. I, fig 1). The coarser fibres appear blue-bordered on both sides, as would be the case if the myelin sheath and not the axon were stained. That the blue stain is not taken up by the axon itself is shown by the pictures of transected coarser fibres, which present themselves as blue rings with no coloration of their central areas. The smallest fibres which possess a double-bordered outline have an outer diameter of about 1μ, and correspondingly the smallest rings are of the same diameter, some even somewhat smaller. In

[1] A male aged 61, death occurring on account of coronary occlusion.

the latter the blue ring surrounding the central pale area is very thin. In addition to these fibres and rings, which are clearly to be interpreted as nerve-fibres endowed with a myelin sheath, there occurs, however, a multitude of finer blue lines and streaks, which even with the highest magnification cannot be seen to present a double blue border-line (Pl. I, fig. 2). Some of these pursue a fairly straight course for some distance, but the majority of them make frequent bends and can consequently in these thin sections be followed as uninterrupted structures only for a relatively short distance. Occasionally they may be seen to branch dichotomously, or to give off side branches, presumably collaterals. Now and then it is also possible to follow the gradual transition of one of the smallest double-bordered fibres into a very thin singly contoured fibre of a diameter of about $0{\cdot}5\mu$. Quite frequently these show smaller swellings, being for a short distance double-bordered, or showing small heavier blue spherules along their course (see Pl. I, fig. 2). The meshes between the nerve-cells of the cortex are partly filled with fibres, predominantly of the smallest calibre and minute rings (cross-sectioned fibres), but in addition there are numerous minute granules, and very short fragments of the tiniest blue fibres. The distinctly outlined minute blue spots are presumably cross-sectioned fibres of the finest type. Their diameter is between 1 and $0{\cdot}5\mu$, some even less, but definite estimation of the thickness is impossible with smaller diameters. The individual nerve-cells are frequently seen to be encircled by some such extremely fine fibres.

The nerve-cells are usually not stained, and have a very faint yellow-white colour. The nucleus is visible when the opening of the diaphragm is reduced, as is also the nucleolus. In some places, however, some of the nerve-cells are shrunken, with definite angles, and have either a dark, dirty yellow colour, or are stained blue-black. These changed nerve-cells correspond to the artificially shrunken cells, which are commonly observed in the brains of animals, mainly near the surface, and which have been studied by several authors. By some their appearance is assumed to be due to mechanical interference in the dissection of the brain, a view which would explain their tendency to occur in superficial parts of the brain and in patches (see e.g. Droogleever Fortuyn, 1927; Scharrer, 1937 and 1938). In some of these cells the apical dendrite, and in others the neurite, can be seen to have acquired a blue colour. The connective tissue of the meninges and blood-vessels is slightly yellow, the red blood corpuscles being a blue to dark blue-black colour.

Mainly similar findings are made in several other parts of the central nervous system of rats, of which only some will be mentioned below.

The Cerebellum. The cortex assumes a bluish colour. When seen with high power, however, there is in most places no clear-cut pattern of the fibres in the molecular layer as seen in silver preparations, but the blue tint is observed to be due to a limited extent only to some very tiny fibres of about $0{\cdot}5\mu$; some of these ascend towards the surface, others are horizontal. In addition very fine blue dots occur everywhere. In some places, however, mainly in superficial parts of the cerebellum, where the Purkinje cells are shrunken (cf. below), a

ultitude of extremely thin tangential fibres is visible (in longitudinal sec-ons of the folia, and therefore mainly neurites from granule cells). Only ery few rings of the smallest type are observed, as evidence of cross-sectioned yelinated fibres. That the finest fibres are not glial fibres is extremely robable from the occurrence of swellings and irregularities in their calibre hich are well known to occur in preparations of myelinated fibres. Just as the cerebral cortex, it may be observed how a single contoured fibre of a ameter of about $0{\cdot}5\mu$ for a short distance is swollen and shows a double-rdered part, similar in appearance to the thicker myelinated fibres else-here. In the granular layer mostly coarser fibres are seen, but quite a mber of smaller ones, usually of an irregular course or transected, are to be und (Pl. I, fig. 5). Between groups of granular cells accumulations of small ue dots occur interspersed with some distinguishable fine fibres, corre-onding, it appears, to the so-called cerebellar glomeruli. It is not possible decide whether these blue patches are only conglomerations of stained res or precipitation artifacts in the matrix (though neither formal calcium r dichromate calcium is a protein precipitant). Many of the Purkinje cell clei are shrunken and stain more or less blue-black. Such cells are found ainly in the superficial folia, less in the depths of the sulci. In some of these lls also the branching dendrite can be followed (and in the same regions the nount of finest fibres is particularly large). It is, however, striking that the re baskets surrounding the Purkinje cells and the supra- and infraganglionic vers of tangential fibres so clearly seen in silver preparations are nowhere sible, only a few medium-sized fibres occurring in these places. The picture the intracerebellar nuclei is clearer; coarser, finer, and finest fibres, longi-dinally or transversely cut, occur in abundance between the nerve-cells. It ould be noticed that the cells of the cerebellar nuclei regularly contain a fair nount of fine blue granules in their cytoplasm; these are frequently arranged rows, like the tigroid granules, but on the whole the size of the granules served in our preparations is finer and they are of more uniform size than e tigroid granules.

The Hypothalamus. This region, commonly regarded as being scanty in yelinated fibres, is distinguished also in the acid haematein preparations by ing less blue than most other regions of grey matter. However, with high wer, quite a number of blue fibres are found, transversely or longitudinally t. Coarser fibres are very rare, and the majority of the fibres observed are the medium and finest type. Also in this region numerous blue fibres cur, with a diameter of less than 1μ down to about $0{\cdot}5\mu$, and ring-like uctures and dots of corresponding size are frequent. The medial fore-ain bundle can be identified with the naked eye in these preparations by bluish tint.

The Spinal Cord. Similar small fibres are observed as in the other parts. ius, extremely fine fibres can be seen entering the grey matter of the dorsal rns and coursing in a ventral direction, some of these having a diameter of out $0{\cdot}5\mu$, or even less (cf. Pl. I, figs. 3 and 4).

Human Material

In principle the findings are similar to those in the preparations from the rats' brains. However, on gross examination, the grey matter appears less blue than in the sections of the latter, and on microscopical examination the explanation of this difference is obvious. The number of finer fibres acquiring a blue colour is definitely less, and in addition the blue colour of the thicker and medium-sized myelinated fibres is distinctly lighter than in the rats. However, there are also present in the sections of the human brain, for instance in those from the cerebral cortex, small blue rings of a diameter of about 1 μ, and now and then a tiny blue fibre of a diameter of about 0·5 μ is encountered, revealing the same properties as described in the similar fibres in the rat material. Furthermore, the multitude of minute blue dots and fragments of linear structures, to be seen everywhere in the grey matter of the rats' brains, is almost completely lacking in the corresponding areas of the human brain, where the myelinated, coloured fibres stand out against a pale-yellowish background. In other parts of the brain, as for instance the hypothalmus, the cerebellar cortex, and the inferior olives, the number of blue fibres and dots of the smallest size is even less than in the cerebral cortex and almost negligible. Shrunken cells, assuming a dark-grey or blue colour in acid haematein preparations, are not seen in the human brain, in accordance with their regular absence after the usual methods of preparation.

B. *Pyridine-extracted Material*

In the sections from the rat the white matter appears grey with a slightly blue tint. The grey matter acquires a yellow hue. On microscopical examination the cells are seen to be yellow, while the nucleolus and sometimes also some of the chromatin material assumes a dark blue-black colour. Since the findings are essentially similar in all regions of the brain, only a general description of the appearance of the fibres will be given.

The coarser fibres, regardless of whether they occur isolated or in larger bundles, have a greyish colour with a slight blue tint. The blue tint is, on the whole, a little more prominent in the deeper regions than in the superficial, a fact which may possibly indicate that the lipoid extraction has been less complete in the deeper parts of the block than in the more superficial or, since extraction in general seemed thorough throughout the block, that these fibres contained either more acidic protein, or a protein–lipine complex from which the lipine could not be extracted. Apart from this change in colour as compared with the acid haematein material, the appearance of the myelin sheaths is more diffuse, they are less clearly seen as individual units, less sharply outlined, and the details in the areas composed largely of myelinated fibres on this account appear somewhat indistinct. In cross-sections of such areas, the myelin sheaths are seen as rings, but these are thinner than when seen in acid haematein preparations, and their outline is also less distinct. Their more irregular shape, sometimes wrinkled, may be due to the pyridine-extraction fixative which is a strong protein precipitant. Quite frequently

mall or minute dark-blue spots occur, almost like pearls, along the course of ne myelin sheaths, possibly indicating a deficient extraction of lipine or, more robably, that the precipitating action of the fixative has produced granules of rotein just as it does in nuclei.

Smaller fibres are also to be seen in fair numbers, a few of them even of the ame small size as in the acid haematein sections, down to a diameter of bout 0·5 μ. Likewise rings of a diameter of about 1 μ can be encountered. Iowever, these are less distinct than in the acid haematein sections, perhaps o some extent on account of their more greyish colour. When the richness of nest fibres is compared with that seen in the acid haematein sections, it ppears that their amount is less in the pyridine-extracted material. This is articularly conspicuous in the cerebral cortex, where comparisons are most asily made. Only occasionally in the pyridine-extracted sections are finest ibres observed in the outer half of the thickness of the cortex, whereas such ibres were found consistently also in the superficial parts of the cortex in the cid haematein material. Similar conditions prevail in the cerebellar cortex. The amount of stained fibres in the granular layer is less, and the molecular ayer is practically devoid of any stained fibres. However, in parts of all ections there is a considerable number of clear, blue, small rings in the mole- ular as well as in the granular layer, of the type interpreted in the acid aematein sections as cross-sectioned fine myelin sheaths. Along the finest isible fibres pure-blue spots also occur quite frequently.[1]

In the pyridine-extracted sections the red blood corpuscles are stained lue-black, and the nuclei of cells of the connective tissue like those of the ervous tissue are conspicuous by their darkly stained nucleoli; sometimes lso granules of chromatin material are visible.

As in the acid haematein-stained sections, the nerve-cells in several places re shrunken in the pyridine-extracted material. These shrunken cells are arker, dirty grey or slightly blue. It is worthy of mention that in some of the hrunken Purkinje cells, their dendrites with several arborizations are stained clear blue and stand out very conspicuously.

The pyridine-extracted material from the human brain is, in principle, imilar to the rat material, but the difference between the pyridine-extracted ections and the acid haematein-stained sections is less evident. The myelin heaths of the coarser and medium-sized fibres are greyish and less heavily

[1] It should be mentioned that in the sections from one of the pyridine-extracted blocks of rat brain numerous peculiar blue particles were seen, irregularly dispersed throughout the ections, mainly in the grey matter. These minute particles, mostly having a diameter no rger than 1–2 μ, are spherical, ovoid, or comma-shaped and occur sometimes in irregular onglomerations, sometimes isolated or only a few together. It has not been possible to dentify them with either nucleoli, nerve-fibres, glial fibres or intracellular granules, or any ther known structures of the nervous system. No explanation can be offered for their ccurrence only in this block. A slight difference in the treatment of this block, although not ossible to ascertain at the time when the sections were examined, might possibly have ccurred and might explain the discrepancy. Assuming that the methods are specific, as resupposed above, it appears likely that these granules may chemically be of protein ature.

coloured than in the acid haematein sections. However, also in the pyridine-extracted sections a certain number of the finest fibres still stand out by their grey-blue colour, and likewise some minute rings of a diameter of about 1 μ are present. A small quantity of fibres of a diameter of about 0·5 μ can be ascertained, their number being, it appears, a little less than in the acid haematein sections.

C. *Sudan Black and Nile Blue Preparations*

In sections of the rat brain stained with sudan black the white matter acquires a grey to grey-black colour, according to the thickness of the section and the abundance of myelinated fibres. The grey matter is slightly grey when viewed macroscopically. On microscopical examination the myelin sheaths of the coarser fibres acquire an appearance essentially similar to that seen when other myelin-sheath stains are employed, the sheaths colouring more or less black. The same applies to the medium-sized myelinated fibres, which appear double-bordered (Pl. I, fig. 6). With oil immersion lens, smaller fibres with a diameter of 1 μ or somewhat less are also seen, similar to those observed in the acid haematein sections, and rings of the same diameter occur, but, on the whole, their amount is somewhat less and only a few of the very finest fibres can be recognized. Their nature as nerve-fibres is revealed by the fact that they frequently show small double-bordered parts along their course, representing apparently the commonly observed swelling of the myelin sheath. However, these finest fibres stand out somewhat less clearly than in the acid haematein preparations, partly because the greyish colour offers a less favourable contrast to the background than does the pure-blue coloration in the latter sections, and partly because the acid haematein test is more sensitive. It is difficult to decide whether the numerous tiny grey dots in the grey matter, e.g. in the cerebral and cerebellar cortex, represent extremely fine fibres or fragments of fibres in cross-section or not, but this interpretation is favoured by the observation of small linear grey structures, reminiscent of fibres, which are sometimes seen to arborize.

The nerve-cells are usually unstained, or, particularly in the larger cells, their cytoplasm has a faint greyish hue. The artificially shrunken cells assume a darker grey colour of the cytoplasm, the nucleus being colourless.

In nile blue preparations the more heavily myelinated fibre tracts stand out clearly, owing to their pure, light-blue colour. Examined with the high power of the microscope, the myelin sheaths are seen to take a light-blue stain. As in the other preparations described above, in the nile blue stained sections very fine fibres can also be seen, but it is difficult to judge their colour when they approach a thickness of 1 μ.

It should be noted that the cytoplasm of many, although not all, nerve-cells stains distinctly blue. On this account these cells are very conspicuous even when the sections are examined with low power, and in those stained more heavily the nucleus is stained a lighter blue. In the artificially shrunken cells the blue staining of the cytoplasm is very intense, and the nucleus is only

lightly lighter than this. No red coloration was seen anywhere in the central ervous system.

Discussion

Before interpreting the findings made in this material, attention should be rawn to some data from the literature relevant to the application of histo-hemical tests to the central nervous system in general. The setting free of hosphorus, probably due to the breakdown of phosphatides, which appears o start immediately after cessation of the circulation of the brain at death Jungmann and Kimmelstiel, 1929), is probably negligible in the rat material, ince the brains were fixed at the most 10 minutes after the animals were illed. In the case of the human brain autolysis may have played a role, owever, for there was an interval of $24\frac{1}{2}$ hours between death and fixation. For this reason, the conclusions drawn from our observations will be mainly ased on the findings in the rats' brains. According to Weil (1929, 1946) a iminution in the amount of phospholipines, and less of galactolipines, ccurs during formalin fixation (the phospholipines being hydrolysed), in-reasing with the duration of the fixation and the acidity of the formalin olution. The formal calcium employed in Baker's method is designed, owever, to eliminate this loss of lipines.

The observations made in this study give some hints concerning the con-titution of myelin sheaths. Baker's (1946) acid haematein test is claimed to e specific for phospholipines: therefore one must presume that our findings ave demonstrated the presence of this substance in the myelin sheath. he pyridine-extraction control test, while eliminating lipines, increases the endency of proteins to give a positive reaction. The finest, and even the yelin sheaths of the coarser, fibres in pyridine-extracted material still ave a slight blue tint: an obvious explanation for this is that the lipoid xtraction has not been complete, although in our material the extrac-on was exactly performed, but the possibility is still not excluded that the rotein in the myelin sheath is being stained. This point will be further iscussed later. Sudan black, after fixation in Baker's (1944) formal calcium, olours all classes of lipoids, and it is quite likely that the phospholipine emonstrated by the acid haematein test does not necessarily constitute all ie lipoid in the myelin sheath as shown by sudan black; thus it is possible iat galactolipines may be present, and therefore no evidence either for or gainst the statement that galactolipines form one-third of the myelin sheath Weil, 1946) has been obtained.

According to Cain (1947*b*), provided a body is known to be lipoid, nile blue in be used to detect acidic lipoids in it; if it consists only of neutral lipoids will be coloured red, if acidic lipoids (i.e. fatty acids and other lipoids ich as lipines, including lecithin and probably other phospholipines which n behave as acids) are present it will be coloured blue. Pending confirmation Cain's results it can therefore be provisionally assumed that acidic lipoids e present in the central nervous system but, although no red coloration

was seen, the presence of neutral lipoids is not excluded since a red coloration is easily masked by a blue. The blue staining of the myelin sheaths with nile blue can be taken to confirm, at least partly, the observations with the acid haematein test.

The considerations set forth above concerning the chemical constitution of myelin and the reliability of the histochemical tests employed make pertinent some discussion of the question of myelinated and unmyelinated fibres in the central nervous system. In the acid haematein preparations of the rats' brains, fibres staining the typical blue-black colour and having a diameter of as little as $0{\cdot}5\,\mu$, or even somewhat less, were seen. Actually this value might be a little higher, on account of the shrinking due to treatment of the tissue, but the percentage of shrinkage is scarcely of a magnitude which fundamentally alters the values as they were measured in the fixed and stained specimens, since formalin causes no shrinkage (see e.g. Rexed, 1944; Baker, 1945) and the usual dehydrating agents are not used in the acid haematein test. These tiny fibres are most clearly seen in the acid haematein sections, somewhat less distinctly and in a smaller amount in the sudan black and pyridine-extracted sections. This might be explained in accordance with the findings of Schmitt and Bear, that the proportion of protein in the myelin sheath increases with decreasing size, but that a certain amount of lipoid is still present in the smallest myelinated fibres. In the acid haematein sections the lipine constituents, or perhaps at times the protein constituents, will contribute to give a blue-black colour with acid haematein. In the sudan black sections only lipoids colour, but the amount of lipoid in sheaths of the smallest fibres may not be sufficient to give a distinct coloration. In pyridine-extracted sections the proteins, possibly together with some bound lipine, are responsible for the staining properties. It seems from these observations, therefore, always providing the acid haematein test is specific, that, since there is lipoid in these small fibres of a diameter of about $1\,\mu$ as shown by sudan black, and since in all larger fibres lipine, as shown by the acid haematein test, is present, the lipoid in the smallest fibres of about $0{\cdot}5\,\mu$ contains some lipine, though this cannot be stated as a direct inference from the results of the acid haematein test, since the protein of the sheath has some tendency to stain blue after pyridine extraction. This, then, would mean that a myelin sheath must be assumed to be present in fibres of a finer calibre than has been shown by previously employed anatomical methods and the methods of polarization optics. The lower limit of the fibre size investigated by Schmitt and Bear appears to be of the order of $1\,\mu$, smaller fibres being apparently impossible to analyse by their technique, owing to the small radii of these sheaths. Histologically some authors report having observed a few fibres in some places of a diameter less than $1\,\mu$, as mentioned in the introduction, but such observations, as far as can be seen, appear to be isolated. Usually the limit between myelinated and unmyelinated fibres is drawn at a thickness of $2\,\mu$, fibres with a diameter below this value being considered as unmyelinated (see e.g. Duncan, 1934). It appears that neurophysiologists generally

ccept this limit, and that the recording of electrical changes in fibres f a smaller diameter is beset with great difficulties (see e.g. Zotterman, 939).

In this study we have confined ourselves to the central nervous system, and ne primary object of the investigation was to try to obtain information con- erning the chemical composition of the myelin. We have not been able to iscover any studies in the literature on the subject of the smallest size of nyelinated fibres present in the central nervous system, whereas this problem as aroused great interest with regard to the peripheral nervous system. The nost recent work on the calibre of fibres of the central nervous system ap- ears to be that of Szentágothai-Schimert (1941), but this author considers bres with a diameter of less than 2μ collectively, and no data on the exact ize of the smallest fibres present are given. Nor does Häggqvist (1936) give letails in this respect in his study of the fibres of the spinal cord of man, lthough he mentions that a certain number of fibres thinner than 1μ are resent. Since, however, Häggqvist employed a modified Alzheimer-Mann echnique which according to Rexed (1944) causes considerable shrinkage some 27 per cent.), his figures cannot be taken to indicate the presence of bres thinner than 1μ having a myelin sheath. The data for comparison nentioned in the introduction are therefore, as will be seen, all from studies f the peripheral nervous system, and our results do not necessarily imply hat the acid haematein test will be able to show the presence of similarly ne myelinated fibres in the peripheral nerves or autonomic system, although his appears likely. It may be mentioned in this connexion that in well- tained Weigert-Pal sections of the central nervous system of man, occasion- lly a fine myelinated fibre of a diameter less than 1μ can be encountered. The contrast between this finding and the ample occurrence of these and ner fibres in our rats' brains is probably to be explained by a partial disinte- ration of the lipoids in the human brain between the time of death and utopsy, partly also by the influence of the formalin fixation (cf. above). The carcity of such fine fibres in the human brain studied by us with the acid aematein test is probably only due to the former factor, since formal- alcium was used for fixation. It is, however, likely that similar fine myeli- ated fibres are present also in the human brain, but they escape recognition hen some time elapses between death and fixation. The lack of the minute lue dots and linear fragments, so universally present in the grey matter f the rats' brains, is in accord with this view, as is also the lighter colour ob- erved in the thicker myelinated fibres in the human brain. In order to test he validity of these assumptions the brain of a rat was fixed $17\frac{1}{2}$ hours after he animal was killed, and stained with acid haematein. These sections are ery similar to those from the human brain. Although occasionally a very few f the finest fibres are seen, the multitude of such fibres and minute blue dots resent in those rat brains fixed immediately is completely lacking, and the con- ast between the two is very striking.

If the observations made in this study are taken to indicate that fibres of a

diameter as small as 0·5μ possess a lipoid investment, a myelin sheath, the range of the myelinated fibres is made to extend over practically the entire fibre spectrum, and the observations lend support to the now generally held view that there is no significant qualitative difference between myelinated and so-called unmyelinated fibres. Even if it is not possible to measure fibres of a diameter of less than 0·5μ with any degree of accuracy, it appears from our sections that there is probably a considerable quantity of even finer, extremely thin fibres of the same type in the central nervous system. The minute blue dots and tiny linear fragments which are seen everywhere in the grey matter of the rat brain, as described above, are probably such fine fibres. That they are glial fibres appears extremely unlikely, since they betray no relation to the glial nuclei and the pictures do not resemble those given by the specific glia staining methods. These observations are most readily made in the cerebral and cerebellar cortex of the rat's brain. This multitude of finest fibres, then, would probably correspond to the so-called neuropile. Whether the interpretation given here is correct remains to be shown by further research, but if so it would mean that even the finest branches are not devoid of a lipoid investment.

However, even if our observations are taken to show that fibres of a diameter of 0·5μ or less have a lipoid investment, they do not prove that this applies to *all* such fibres. The existence of true unmyelinated fibres is, of course, not excluded by our findings. Attention should be drawn to some observations bearing on this question. In some places, particularly in the Purkinje cell layer and molecular layer of the cerebellum, the number of medium-sized fibres seen in acid haematein sections is certainly less than that shown by a successful silver stain, for example, the clear-cut picture of the baskets surrounding the Purkinje cells not being seen in our preparations. They were also not visible in either pyridine-extracted or sudan black sections. Since many of these fibres are not particularly fine, these observations mean either that these fibres contain so little lipoid that they cannot be detected by these methods or that they are virtually unmyelinated fibres. It should be remembered, however, as is well known, that the silver methods are not histochemically specific. The same applies to the Weigert-Pal method, and herein lies its difference from the acid haematein test.

It has been mentioned that in both acid haematein and pyridine-extracted sections of the rats' brains some of the nerve-cells and their processes are shrunken and stained blue while others are unstained. The explanation for this is most probably that those cells and fibres which are stained have a different chemical constitution from those that are unstained. In the regions where these shrunken cells are most abundant the amount of stained fibres is also much more profuse than usual. This does not mean, of course, that such chemical differences are present in life, for they are probably made obvious by local injury to the cells on removal of the brain. The blue staining is due to either protein or protein-bound lipine in the pyridine-extracted sections, and its presence only in some cells may be therefore due to injury to some

cells and not to others with consequent differential cellular fat or protein phanerosis.

Summary

Baker's (1946) acid haematein and pyridine-extraction control tests, claimed to be specific for phospholipines (Baker, 1947), have been applied to various parts of the central nervous system of rats and man. The sudan black method for the detection of lipoids and the nile blue method for the staining of acidic lipoids have also been used.

The findings are in agreement with older statements in the literature that myelin contains a considerable amount of phospholipines. It was impossible to determine whether galactolipines or neutral lipoids are also present.

In the acid haematein-stained sections finer fibres were seen than when other stains for myelin sheaths are employed. Fibres with a diameter of $0{\cdot}5\,\mu$ or even somewhat less were stained in various parts of the central nervous system of rats. It is regarded as probable from these findings that fibres down to $0{\cdot}5\,\mu$ or even smaller possess a lipoid investment. These observations lend support to the now commonly accepted view that the distinction between myelinated and so-called unmyelinated fibres is arbitrary. Some observations are made, however, which indicate that the presence of truly unmyelinated fibres cannot be excluded.

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REFERENCES

Baker, J. R., 1944. Quart. J. micr. Sci., **85**, 1–71.

—— 1945. *Cytological Technique*, 2nd ed. London (Methuen).

—— 1946. Quart. J. micr. Sci., **87**, 441–70.

—— 1947. Ibid., **88** (in the press).

Bielschowsky, M., 1935. Allgemeine Histologie und Histopathologie des Nervensystems, in Bumke, O., and Foerster, O., *Handbuch der Neurologie*, vol. i (1), p. 84. Berlin (Julius Springer).

Blair, E. A., and Erlanger, J., 1933. Amer. J. Physiol., **106**, 524–64.

Cain, A. J., 1947*a*. Quart. J. micr. Sci., **88**, 151.

—— 1947*b*. Ibid., **88** (in the press).

Droogleever Fortuyn, A. B., 1927. J. Comp. Neur., **42**, 349–91.

Duncan, D., 1932. Ibid., **55**, 459–71.

—— 1934. Ibid., **60**, 437–62.

Foley, J. O., and DuBois, F., 1943. Ibid., **79**, 79–105.

Grundfest, H., and Gasser, H. S., 1938. Amer. J. Physiol., **123**, 307–18.

Häggqvist, G., 1936. Z. mikr.-anat. Forsch., **39**, 1–34.

Harrison, R. G., and Cain, A. J., 1947. J. Anat., Lond., **81**, 286–99.

Hillarp, Nils-Åke, and Olivecrona, H., 1946. Acta Anatom., **2**, 17–32.

Jungmann, H., and Kimmelstiel, P., 1929. Biochem. Z., **212**, 347–62.

Kiss, F., and Mihalik, Peter v., 1929. Z. Anat. Entw. Gesch., **88**, 112–51.

Koch, W., and Koch, M. L., 1913. J. biol. Chem., **15**, 423–48.

Page, I. H., 1937. *Chemistry of the Brain*. London (Baillière, Tindall & Cox).

Ranson, S. W., Droegemueller, W. H., Davenport, H. K., and Fisher, C., 1935. Res. Publ. Ass. Nerv. Ment. Dis., **15**, 3–34.

Rexed, B., 1944. Acta Psychiat. et Neurol., Suppl. 33.
Scharrer, E., 1937. Beitr. z. Pathologie, **100,** 13–18.
—— 1938. Anat. Rec., **72,** 53–65.
Schmitt, F. O., and Bear, R. S., 1937. J. cell. comp. Physiol., **9,** 261–73.
—— —— 1939. Biol. Rev., **14,** 27–50.
Szentágothai-Schimert, J., 1941. Z. Anat. Entw. Gesch., **111,** 201–23.
Weil, A., 1929. J. biol. Chem., **83,** 601–9.
—— 1946. *Textbook of Neuropathology,* 2nd ed. London (Heinemann).
Zotterman, Y., 1939. J. Physiol., **95,** 1–28.

EXPLANATION OF PLATE

Fig. 1. Microphotograph from rat's cerebral cortex. Acid haematein stain, × 500. Medium-sized and small myelinated fibres are seen between the cells. Minute fibres and blue dots account for the darkness of the background.

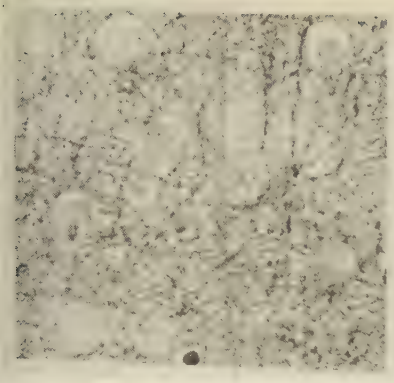
Fig. 2. Detail from cerebral cortex of rat. Acid haematein stain, × 1,000. Some of the finest fibres with a diameter of about 0·5 μ are indicated by arrows; others, as well as several of the minute dots and tiny fibre fragments, are out of focus (cf. text).

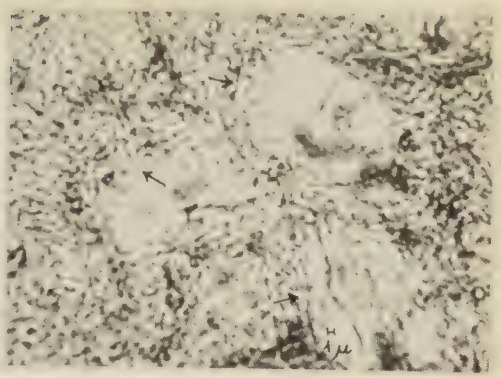
Fig. 3. Microphotograph from the dorsal horn of rat's spinal cord, showing several fine fibres staining in acid haematein sections. Some of the fibres with a diameter of about 0·5 μ are indicated by arrows. × 500.

Fig. 4. Detail from the centre of Fig. 3, showing the branching fibre with higher magnification, × 1,800. The irregular calibre characteristic of myelinated fibres as seen in histological preparations is visible.

Fig. 5. Microphotograph from the granular layer of the cerebellar cortex of the rat. Acid haematein stain, × 1,000. Coarser, medium-sized, and very fine fibres are seen between the granular ceils. Some of the finest fibres, *c.* 0·5 μ, are indicated by arrows.

Fig. 6. Microphotograph from the vestibular nuclei in a sudan black stained section of the rat's medulla. × 1,800. In addition to thicker myelinated fibres smaller fibres have also taken the stain.





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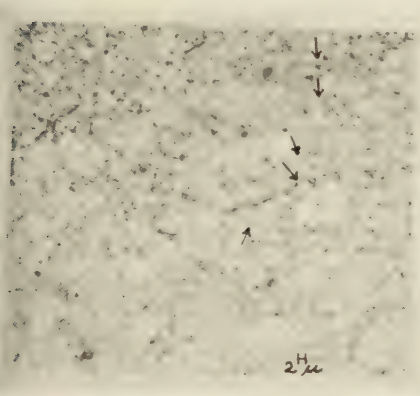

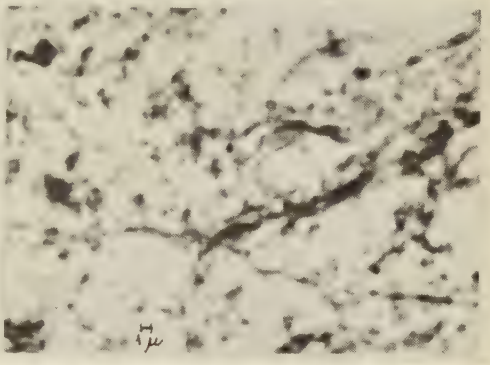

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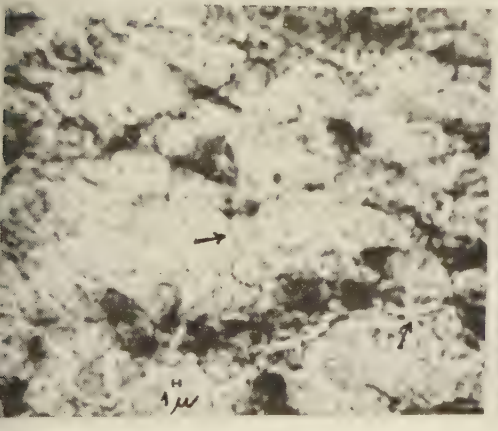

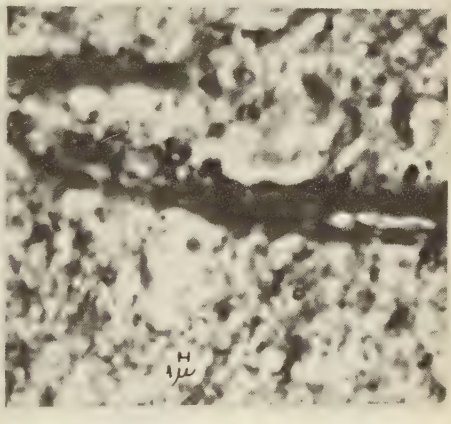

6

A. BRODAL AND R. G. HARRISON.—PLATE I

The Cell-theory: a Restatement, History, and Critique

PART I

BY

JOHN R. BAKER

(From the Department of Zoology and Comparative Anatomy, Oxford)

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INTRODUCTION

SEVERAL zoological text-books published during the last two decades have cast doubts on the validity of the cell-theory. These books do not present in any comprehensible form the evidence on which the doubts are based. I therefore set myself the task of finding and studying the evidence, in order to be able to form some judgement of its weight. When the evidence was examined, it became apparent that different parts of the theory were being attacked, and that one attack might be justifiable while another was not.

Now there is no generally accepted body of opinion as to what the cell-theory is. The phrase 'cell-theory' was invented by Schwann, who has told us what he meant by it (1839*a*, p. 197). 'One may include under the name of *cell-theory*, in the wider sense,' he wrote, 'the exposition of the statement that there exists a general principle of construction (Bildungsprinzip) for all organic products, and that this principle of construction is cell-formation.' Unfortunately, the word Bildungs introduces uncertainty into the meaning of Schwann's definition, for it may refer either to *structure* or to *development of structure*. He seems to have meant the latter. Schwann thought that the development of structure took place in two stages: the development of cells from a structureless substance, and the differentiation of those cells into their definitive forms. He expressed this best in a little-known passage (Schwann, 1839*b*, p. 139), contributed to a work by another author. He here gives a clearer and better definition of the cell-theory than in his celebrated book.

'A common principle of development (Entwickelungsprinzip)', he wrote, 'is the basis of all organic tissues, however diverse they may be, namely, cell-formation (Zellenbildung); that is to say, nature never joins the molecules together directly into a fibre, tube, &c., but always first fashions a cell or first transforms this cell, where necessary, into the different elements of structure as they occur in the adult state.'

Schwann's opinion as to the first stage of the development of structure was quite wrong. In October 1837 he took over from Schleiden, in conversation (Schwann, 1839*a*, p. x; 1884, p. 25), an entirely false theory as to how cells are formed. Following Schleiden, he called the nucleus a Cytoblast or cell-bud. He thought that nuclei were formed by a process resembling crystallization in a structureless fluid, the Cytoblastem (or cytoblastema, as it is often spelled in English); that each secreted a membrane round itself; and that what we nowadays call the cytoplasm then appeared within this membrane. The method by which cells are formed, however, was not a subject that Schwann investigated with any thoroughness: his research was devoted to the structure of cells, rather than to the process by which they originate. For instance, the cartilage-cell was one of his first objects of study in connexion with the cell-theory (Schwann, 1838*a*), and he laid especial stress on it in his book (1839*a*); yet his evidence for the view that it develops from a Cytoblastem in accordance with Schleiden's scheme is wholly indirect. This is made particularly clear in the English edition of his book (1847), for which he rewrote part of the section dealing with cartilage. Nevertheless, Schwann not only believed that animal cells resemble those of plants in their mode of development from a structureless Cytoblastem, but thought that it was he who had discovered this, and insisted on his priority in the matter in the course of an argument with Valentin, attached to the end of his book in both editions.

Schwann drew a sharp distinction between his cell-theory (Zellentheorie) and his theory of the cells (Theorie der Zellen). The former he regarded as inductive, the latter as speculative. In his theory of the cells he is concerned with teleology, with what we should nowadays call the colloid chemistry of cells, and with cellular in relation to organismal individuality. Schwann himself did not regard the last-named subject as part of his cell-theory.

Remak (1855, p. 164), who attributed the cell-theory to Schleiden, defined it as 'the theory of the formation of plants exclusively from homologous components which develop in different ways'. He thus laid stress on the conformity of all plant cells with one another, rather than on the particular way in which they develop. Virchow (1859, p. 9), who was well aware that cells originate from pre-existing cells and who coined his famous aphorism to call attention to this fact, took the phrase 'cell-theory' to mean precisely the erroneous views of Schleiden and Schwann as to their origin.

Other authorities have laid particular stress on the double individuality that they believe to be characteristic of many-celled organisms. Schleiden himself (1838, p. 138) wrote: 'Now each cell leads a double life: the one wholly

ndependent, only connected with its own development, and the other remote, n so far as it has become an integral part of a plant.' Hertwig (1893, p. 3), in defining the cell-theory, added this idea of individuality to a clause that is reminiscent of Schwann and Remak: 'Animals and plants, so diverse in their external appearance, agree in the fundamental nature of their anatomical construction; for both are composed of similar *elementary units*, which are generally only perceptible under the microscope. Through the influence of an old theory, now discarded, these units are called cells, and thus the doctrine that animals and plants are composed in an accordant manner of very small particles of this kind is called the *cell-theory* . . . the common life-process of a composite organism appears to be nothing else than the exceedingly complicated result of its numerous and diversely-functioning cells.' Bourne (1895, p. 162) also seems to be influenced by Schleiden's ideas when he sums up the cell-theory thus: 'The multicellular organism is a colony, consisting of an aggregation of separate elementary parts, viz. cells. The cells are independent life units, and the organism subsists in its parts and in the harmonious interaction of those parts.' (Bourne himself only accepted a part of this theory as true.) In recent times, Karling (1939, p. 525) has expressed himself similarly: 'The concept of the organism as a mass of cells which integrate and interact to form a co-ordinate whole is perhaps the real climax of the theory.' Other authors have regarded the homology of the cells of many-celled organisms with individual protists as an essential part of the cell-theory. This opinion as to the meaning of the theory has been held by some of those who attack it (e.g. Dobell, 1911). Indeed, the attack on the cell-theory has been mainly directed against this aspect of it.

The diversity of views about the meaning of the expression 'cell-theory' makes it evident that the truth or untruth of the theory can only be established if it can be formulated in clear terms. The different parts of the cell-theory do not necessarily stand or fall together, and the formulation should therefore be in a series of separate propositions, each of which can be examined independently. No proposition which was formerly thought to be a part of the cell-theory, but which is universally discarded by modern biologists, need be included in the formulation; for instance, it would be useless to set up Schleiden's view as to the mode of origin of cells and then demolish it.

Bearing these considerations in mind, I restate the cell-theory in a series of seven propositions, as follows:

I. Most organisms contain or consist of a large number of microscopical bodies called 'cells', which, in the less differentiated tissues, tend to be polyhedral or nearly spherical.

II. Cells have certain definable characters. These characters show that cells (*a*) are all of essentially the same nature and (*b*) are *units* of structure.

III. Cells always arise, directly or indirectly, from pre-existing cells, usually by binary fission.

IV. Cells sometimes become transformed into bodies no longer possessing all the characters of cells. Cells (together with these transformed cells, if present) are the living parts of organisms: that is, the parts to which the synthesis of new material is due. Cellular organisms consist of nothing except cells, transformed cells, and material extruded by cells and by transformed cells (except that in some cases water, with its dissolved substances, is taken directly from the environment into the coelom or other intercellular spaces).

V. Cells are to some extent individuals, and there are therefore two grades of individuality in most organisms: that of the cells, and that of the organism as a whole.

VI. Each cell of a many-celled organism corresponds in certain respects to the whole body of a simple protist.

VII. Many-celled plants and animals probably originated by the adherence of protist individuals after division.

In considering the evidence for and against each proposition, it was necessary to discover how the various bodies of opinion originated and developed. The history of the cell-theory has been told many times, not only in the indispensable standard text-books of the history of biology, but also in other books and in papers. The historical studies that have been of particular use to me are those of Burnett (1853), Tyson (1870 and 1878), M'Kendrick (1888), Turner (1890 *a* and *b*), Sachs (1890), Hertwig (1893), Karling (1939), and Wilson (1944); those of Mark (1879–80), Strasburger (1880), Waldeyer (1888), Rádl (1930), Conklin (1939), and Woodruff (1939) have also been helpful. Among all the existing literature, however, I have not found a sufficiently detailed or accurate study of the origin and development of the various bodies of opinion. What has been written previously on the subject has helped me mainly by leading, directly or indirectly, to original sources. I have not relied and shall not rely in a single instance on a statement by one author as to what another says. A considerable amount of research has been necessary, which has shed new light on some parts of the cell-theory and contradicted certain generally accepted opinions.

In seeking to affect opinion, scientists are usually careful to make their conclusions verifiable with the least possible trouble. It would be a pleasure to be able to say the same of historians of science, but unfortunately it would also be an unpardonable exaggeration. I am creating something of a precedent by giving exact page-references to most of the sources of my information, except when they are contained in such short papers that this is unnecessary. I intend in this way to make it as easy as possible for readers to check the accuracy of what I say and to correct any errors. All verbal quotations given in this series of papers will be rendered in English. The exact references will make it easy to find the originals in every case. In translating from the various languages, I have tried to be as literal as is consistent with the writing of genuine English: no attempt has been made to preserve any foreign

grammatical forms and thus produce the kind of half-translation that is familiar in scientific literature. I have not relied on the translations of others, except where the author's manuscript was translated before publication, or where the original was not available to me for some other reason. In the case of Swammerdam's *Biblia naturae* (1737–8), which was written by the author in Dutch but printed in Latin and Dutch in parallel columns, I have used the Latin version (except where the contrary is stated). When a printed original source has not been available, I have made it clear that the translation used was not my own, and have given a reference to the translation instead of to the original.

The books used in this investigation have been provided by the Radcliffe and Bodleian libraries and those of the Department of Botany at Oxford, of the Royal Society, the Royal Geographical Society, the British Museum, and the British Museum (Natural History).

I hope that readers who may disagree with the conclusions I draw as to the validity or invalidity of the several propositions may, nevertheless, find some value in the historical parts of this series of papers.

The attempt will be made to express all ideas with the utmost clarity and simplicity, so that there will be no mistake about my meaning. Then, if the ideas are wrong, they can easily be corrected.

In composing the papers I have received help from the criticisms of the cell-theory by Whitman (1893), Sedgwick (1894 and 1895), Bourne (1895, 1896 *a*, *b*, and *c*), Awerinzew (1910), Dobell (1911), Baitsell (1940), Weiss (1940), and Ries (1943). I acknowledge the assistance given by friends in the course of informal discussions. In particular I must mention the encouragement given by Prof. A. C. Hardy, F.R.S., which has helped me in a long and rather difficult task, somewhat removed from the main stream of modern cytological advance.

Proposition I

Most organisms contain or consist of a large number of microscopical bodies called 'cells', which, in the less differentiated tissues, tend to be polyhedral or nearly spherical.

The Discovery of Plant Cells

Strangely enough, the earliest published account of the microscopical structure of plant tissues is concerned with petrified wood. In describing the resemblances of this material to ordinary wood, Hooke refers to the 'conspicuous *pores*'. He continues: 'Next (it resembled wood) in that all the smaller and (if so I may call those which are only to be seen by a good glass) *microscopical* pores of it, appear (both when the substance is cut and polish'd *transversly*, and *parallel* to the pores) perfectly like the *Microscopical* pores of several kinds of *wood*, retaining both the shape, and position of such pores.' The 'conspicuous *pores*' may have been either resin-canals or large vessels. Most of the '*microscopical* pores' were presumably small vessels, though some

of them may have been cells of the medullary rays or of the wood parenchyma. Hooke regarded them as tubular. He contributed his account of the structure of petrified wood to Evelyn's *Sylva* (1664, p. 96).

Evelyn spells Hooke's name 'Hook', but there is no doubt about the identity of the famous microscopist, for Hooke refers to his study of petrified wood again in his *Micrographia* (1665, p. 100). In this work, before describing cork, Hooke writes '*Of* Charcoal, *or burnt* Vegetables.' In describing the stems of plants, burnt to charcoal and broken across, he first notices the large vessels and proceeds (p. 101): 'But this is not all, for besides those many great and conspicuous irregular spots or pores, if a better *Microscope* be made use of, there will appear an infinite company of exceedingly small, and very regular pores, so thick and so orderly set, and so close to one another, that they leave very little room or space between them to be fill'd with a solid body, for the apparent *interstitia*, or separating sides of these pores seem so thin in some places, that the texture of a Honey-comb cannot be more porous. Though this be not every where so, the intercurrent partitions in some places being very much thicker in proportion to the holes.

'Most of these small pores seem'd to be pretty round, and were rang'd in rows that radiated from the pith to the bark.' He considered the 'pores' to be longitudinal tubes. He calculated that there are 2,700 of them to an inch (in a transverse section). He mentions that in sound wood the microscopical pores 'are fill'd with the natural or innate juices of those Vegetables' (p. 108).

After dealing with charcoal and repeating his already-published observations on petrified wood, Hooke comes to his well-known study '*Of the* Schematisme *or* Texture *of* Cork'. He appears to have seen cells in cork before he studied petrified wood and charcoal, for he writes (pp. 112–15) that the cells of cork 'were indeed the first *microscopical* pores I ever saw, and perhaps, that were ever seen, for I had not met with any Writer or Person, that had made any mention of them before this'. He describes how he cut a very thin section of cork and examined it on a black plate with a plano-convex lens. '. . . I could exceeding plainly perceive it to be all perforated and porous, much like a Honey-comb, but that the pores of it were not regular; yet it was not unlike a Honey-comb in these particulars . . . these pores, or cells, were not very deep, but consisted of a great many little Boxes. . . . Nor is this kind of Texture peculiar to Cork onely; for upon examination with my *Microscope*, I have found that the pith of an Elder, or almost any other Tree, the inner pulp or pith of the Cany hollow stalks of several other Vegetables: as of Fennel, Carrets, Daucus, Bur-docks, Teasels, Fearn, some kinds of Reeds, &c. have much such a kind of *Schematisme*, as I have lately shown [in] that of Cork.' Hooke thought that the 'pith' of the shaft of a feather had a similar structure.

Unlike some of his followers, Hooke did not concentrate the whole of his attention upon the cell-wall. He wrote (p. 116): '. . . in several of those Vegetables, whilst green, I have with my *Microscope*, plainly enough dis-

cover'd these Cells or Poles [misprint for Pores] filled with juices . . . as I have also observed in green Wood all those long *Microscopical* pores which appear in Charcoal perfectly empty of any thing but Air.' This passage provides the earliest mention of the substance of cells (as apart from that of cell-walls), though Hooke naturally did not distinguish between cell-substance and sap.

Grew made a study of the microscopical structure of plants independently of Hooke, and saw the cells. He had finished the 'composure' of his little book (Grew, 1672) when Hooke communicated his observations to him. Grew had supposed that the pith of plants consisted partly of cells like those of a honeycomb and partly of long tubes. Hooke now corrected him on this matter and the correction was accepted (p. 78). Even in this little book, Grew carried the study of plant cells much farther than Hooke. He showed (pp. 78–9) the cellular nature of the cortex as well as the pith and illustrated his findings by a figure (his Fig. 15) of the cortex of the stem of the burdock (*Arctium*) in section. This is probably the earliest-published figure of cells as they occur in a living plant (for Hooke's figures were of petrified wood, charcoal, and cork). Grew carried his researches into a still more important field when he demonstrated the cellular nature of plant embryos. He describes the 'sameness' of the nature of the pith and cortex 'with the *Parenchyma* of the Seed. For, upon farther enquiry with better Glasses, I find, that the *Parenchyma* of the *Plume* and *Radicle*, and even of the *Lobes* themselves, though not so apparently, is nothing else but a Mass of Bubbles' (p. 79).

Ten years later, Grew (1682) published his great book on *The Anatomy of Plants*. The figures, many of them representing microscopical dissections in three dimensions like a modern stereogram, are magnificent. In this work he uses the words 'Bladders', 'Cells', and 'Pores' indiscriminately (see, for example, p. 64). He had now acquired, however, a wrong idea of the units of plant structure. His 'Mass of Bubbles' was a much better analogy than that of '*fine Bone-Lace*' (p. 121), which he develops in a celebrated passage in his later work. This subject will be reviewed under the heading of the Proposition II in the second part of this series of papers.

Meanwhile, Malpighi had been busy with the same subject. At the end of his work, *Anatomes plantarum idea*, he wrote the date 1671. In this paper, which is not illustrated, Malpighi calls cells 'utriculi' and 'sacculi'. The *Idea* was first published four years after it was written, in the large volume, *Anatome plantarum* (Malpighi, 1675). This book included more detailed studies of microscopical plant anatomy. The cellular structure of plants is illustrated by figures of transverse and longitudinal sections of stems. These figures do not approach those of Grew (1682) in elaboration of detail. There has been some discussion as to whether Grew or Malpighi had priority in the microscopical study of plant tissues, and whether Grew was more indebted to Malpighi or vice versa. Schleiden (1849, p. 37) was in error when he said that Malpighi despatched his *Idea* to the Royal Society in 1670. He did not do so until November of the next year. Meanwhile, on 1 May 1671

the Royal Society had already given the order for the printing of Grew's little book (1672). Pollender (1868), who has carefully studied the question of priority, awards it unhesitatingly to Grew (p. 7), while allowing that Malpighi's early work was done quite independently.

Leeuwenhoek was not slow to follow up the work of these early investigators of plant cells, to whom he refers, in an undated letter to Hooke, as 'acutissimos Viros *Malpighium* & *Nehemiam Grew*' (see Leeuwenhoek, 1722, p. 13). Leeuwenhoek here gives a large drawing of part of a transverse section through a stem of oak. His writings on plant cells, however, will not bear comparison with those of Grew, and scarcely with those of Malpighi. On this subject he exhibits to the full his character of dilettante of genius. He repeatedly describes and figures the cells of plants, and continues to do so up till near the end of his life. In a letter written in 1713, for instance, he figures them in the seeds of various plants (see Leeuwenhoek, 1719, pp. 25–6). From his time onwards it was a matter of common knowledge among botanists that plants were constructed largely of microscopical chambers, though vessels were not known to be of cellular origin until much later. (The cellular origin of vessels will be discussed under the heading of Proposition IV, in a later paper in this series.) The following statement by Moldenhawer (1812, p. 86) is representative of the best opinion of his time: '. . . the cellular substance thus consists of single, closed, spherical, oval or more or less oblong, almost cylindrical utricles, which, owing to mutual pressure on one another, assume an angular and flattened form, either regular or more or less irregular, and resembling the cells of a honeycomb.'

The Discovery of Blood Corpuscles

The absence of thick cell-walls in most animal tissues put zoologists at a great disadvantage, in comparison with botanists, in recognizing the cellular nature of organisms. Complete separateness of cells was the next best help to microscopists after the presence of easily visible cell-walls. The blood is the only part of an animal that can be compared with most plant tissues in the ease with which it reveals its cellular nature.

It will probably never be known with certainty by whom blood corpuscles were discovered. They were certainly seen by Swammerdam, who died in 1680. It is well known that his *Biblia naturae* was published for the first time long after his death. In this great work (Swammerdam, 1737–8, vol. 1, p. 69), he first mentions blood corpuscles in connexion with his description of the dissection of the louse (*Pediculus*): 'If we begin the dissection in the upper part of the abdomen, and cautiously split the skin there, blood immediately escapes from that place. The blood, when received into a glass tube and examined with a very good microscope, is observed to consist of transparent globules (globulis), in no way differing from cow's milk, a fact that was discovered a few years ago in human blood also; for this is seen to consist of slightly reddish globules, floating in a clear fluid.' It will be noticed that Swammerdam does not state that it was he who made the discovery in human

blood. He remarks: '. . . I shall not recklessly assert that globules are present in the blood of the louse, for it could easily happen that fat might mix itself [with the blood], and so also might certain fragments of the viscera, damaged [by the dissection]; these certainly consist of a mass, as it were, of globular particles, as I shall show at the proper time.' He illustrates the globules, as seen within the glass tube, in Fig. 1 of Tab. II. He did not realize that they might be those of human blood sucked by the louse.

Swammerdam refers to the blood corpuscles of the frog in the second volume of the same book. He writes (p. 835): 'In the blood I saw a watery part, in which floated an immense number of circular particles, rejoicing in a flat, as it were oval, but perfectly regular shape. These particles seemed also to contain another fluid again within themselves. But if I looked at them from the side, they resembled crystalline rods and many other figures; according, doubtless, to the various ways in which they were rotated in the fluid of the blood. I observed moreover that the larger the objects were represented through the intervention of the microscope, the paler their colour appeared [to be].'

Flloyd's translation of the *Biblia* (Swammerdam, 1758) suggests strongly, on p. 120, that Malpighi and Needham already knew of the presence of globules in blood before Swammerdam. The original work was produced in Dutch and Latin in parallel columns. In the passage which Flloyd is here translating, neither the Dutch nor the Latin version gives any evidence for the suggestion that Malpighi or Needham had priority over Swammerdam in this matter.

Unfortunately there would seem to be no means of dating these observations. Foster (1901, p. 99) says that Swammerdam discovered blood corpuscles in 1658 but gives no evidence for this. Miall (1912, p. 198) called attention to the lack of evidence, and no answer appears ever to have been made. It is quite possible that Swammerdam demonstrated the blood corpuscles to the Duke of Tuscany, when the latter visited him and offered him employment. If a record of this particular demonstration exists, the attention of present-day biologists should be called to it. Swammerdam himself (1737–8, p. 839) mentions the Duke's visit, and tells how he demonstrated a nerve-muscle preparation on this occasion. Swammerdam gives 1658 as the date of the visit, but Boerhaave, in writing the great naturalist's biography as a preface to the *Biblia*, states that the date was 1668 (page facing p. C2). Stirling (1902, p. 23), like Foster, gives 1658 as the date of Swammerdam's discovery of blood corpuscles but provides no new evidence.

In his *Exercitatio de omento, pinguedine, et adiposis ductibus*, published in 1665, Malpighi makes the first mention in print of blood corpuscles (see Malpighi, 1686, vol. 2, p. 41). It cannot be claimed that the discovery was made in a satisfactory manner, for he regarded them as globules of fat; yet it is clear from his words that he saw them and that he had no previous knowledge of the existence of such objects in blood. He wrote: '. . . in the omentum of a hedgehog, in a blood-vessel that extended from an accumulation of

fat to another opposite to it, I saw globules of fat, possessing an outline of a particular shape, and reddish; they resembled in general a circle (coronam) of red corals.'

In a letter written to the Royal Society in 1673 (or possibly in 1674), Leeuwenhoek thus describes his own discovery of blood corpuscles (Leeuwenhoeck [*sic*], 1674, p. 23): 'I have divers times endeavoured to see and to know, what parts the *Blood* consists of; and at length I have observ'd taking some Blood out of my own hand, that it consists of small round globuls driven through a Crystalline humidity or water: Yet, whether all Blood be such, I doubt. And exhibiting my Blood to my self in very small parcels, the globuls, yielded very little colour.' Four years later, Hooke (1678, p. 93) attributed the discovery of the 'Globules' of the blood to Leeuwenhoek.

In his characteristically random manner, Leeuwenhoek reverted to blood corpuscles from time to time. In a letter written to the President of the Royal Society in 1683 (three years after Swammerdam's death), he described and figured the red corpuscles of the frog, noting carefully how their colour appears more clearly when two or three are superimposed (see Leeuwenhoek, 1722, pp. 54–5). Writing to the Royal Society again in July 1700, he makes the first mention of the nucleus ('lumen'), and figures it, in describing the blood corpuscles of the salmon and flounder (see Leeuwenhoek, 1719, pp. 219–20). (The discovery of the nucleus will be more fully considered in the discussion of Proposition II.) Writing yet once again to the Royal Society in his old age, in 1717, Leeuwenhoek gives the first indication that human red blood corpuscles are not spheres, but concave disks (Leeuwenhoek, 1719, pp. 421–2). Sixty years later, Hewson (1777, p. 15) observed that mammalian blood corpuscles were not spherical, as was still commonly supposed, but flat, and therefore, he concluded, not fluid.

Although blood corpuscles were discovered at about the same time as the cells of plants, and both soon became familiar objects, it did not occur to the early microscopists that there was any relation between them. It was necessary first to understand that the non-fluid tissues of animals also consisted of immense numbers of minute microscopical bodies. This understanding came, in a roundabout way, through the globule-theory. But before we can follow the tortuous course of progress, it is necessary to clear away a fallacy that has misguided historians of science and given a false impression of the background of the cell-theory.

The 'tissu cellulaire' Fallacy

The expression 'tissu cellulaire', or its counterpart in other languages, occurs frequently in eighteenth- and early nineteenth-century writings on the tissues of animals. Alighting casually upon this phrase, one may easily fall into the error of supposing that it refers to cellular tissue, in the modern sense of the word 'cell'. Historians of the cell-theory, among them Gerould (1922), have been misled by this fallacy. Biologists owe a particular debt to Turner (1890*a*) and Wilson (1944) for calling attention to this matter.

Haller (1757) devotes Sectio II of his *Elementa physiologiæ corporis humani* to what he calls cellular tissue ('Tela cellulosa'). One has only to read what he writes on this subject (p. 9) to realize that he is using the phrase to mean what we should call areolar connective tissue. This appears even more evidently from his 'First lines of physiology' (Haller, 1779). The translator from the Latin of the 1766 edition renders the relevant passage as follows (pp. 3–8):

'The second kind of fibres . . . when loosely interwoven with each other, are called the *cellular* tunic; though the name *tunic* or *membrane* is on many accounts very improper.

'This cellular substance is made up of an infinite number of little plates or scales, which, by their various directions, intercept small cells and web-like spaces; and join together all parts of the human body in such a manner, as not only sustains, but allows them a free and ample motion at the same time. But in this web-like substance there is the greatest diversity, in respect of the proportion betwixt the solid parts and intercepted cells, as well as the breadth and strength of the little plates, and the nature of the contained liquor, which is sometimes more watery, and sometimes more oily. . . . This cellular web-like substance in the human body is found throughout the whole, namely, wherever any vessel or moving muscular fibre can be traced; and this without the least exception that I know of. . . . The principal use of the cellular fabric is to bind together the contiguous membranes, vessels, and fibres, in such a manner as to allow them a due or limited motion. . . . The intervals or spaces betwixt the plates or scales of the cellular membrane, are every where open, and agree in forming one continuous cavity throughout the whole body.'

It is clear that the 'cells' referred to in this passage have nothing to do with cells in the modern sense: they are simply the areolae of connective tissue.

Gerould (1922), who attributes the cell-theory to Lamarck (1809), has been misled by the words 'tissu cellulaire'. There are, indeed, some passages in Lamarck's *Philosophie zoologique* from which one might conclude that he was referring to cellular tissue in the modern sense. For instance, he says 'that the whole operation of nature for the formation of her direct creations, consists in organizing "*en tissu cellulaire*" the little masses of gelatinous or mucilaginous matter that she finds at her disposal and favourable in the circumstances' (1809, vol. 1, p. 373). A careful study of his work leaves no doubt, however, that when he refers to 'tissu cellulaire' in animal tissues, he is never referring to cells in the modern sense, but is nearly always referring to connective tissue. There are a few exceptions. In the case of polyps (vol. 1, p. 203), he appears to use the expression to mean mesogloea, while when he says (vol. 1, p. 210) that infusoria are composed of 'tissu cellulaire', we cannot guess his precise meaning. His words on p. 46 of vol. 2, however, leave no doubt whatever of the usual sense. They must be quoted in full as they settle the matter conclusively:

'. . . all the organs in animals without exception are enveloped in *tissu cellulaire*, and their lesser parts are in the same case.

'In fact, it has been recognized for a long time that the membranes that form the envelopes of the brain, of the nerves, of the vessels of all kinds, of the glands, of the viscera, of the muscles and their fibres, even the skin of the body are generally productions of *tissu cellulaire*.'

Another passage clearly indicating that 'tissu cellulaire' means connective tissue occurs on p. xiv of vol. 1. His repeated insistence (vol. 1, pp. 273 and 409; vol. 2, pp. 47 and 60) that 'tissu cellulaire' is the 'gangue' in which structure is laid down is another pointer in the same direction; for 'gangue' is the substance that encloses a metallic ore in its meshes, not the ore itself. Lamarck's ideas on morphogenesis are so unfamiliar to-day that it is difficult at first to grasp them. He makes himself clearest on this subject on pp. 373–4 of vol. 1. He regarded connective tissue as playing a fundamental part in the development of structure. Fluids move through the meshes of this tissue, and the effect of this movement is 'to open up (frayer) routes . . . to create in it canals, and consequently various organs; to vary these canals and their organs by reason of the diversity either of the movements or of the nature of the fluids'.

When dealing with plants, Lamarck uses the expression 'tissu cellulaire' to mean the cell-walls, or sometimes, more loosely, to mean the cell-walls and their contained fluids. He is therefore thinking of cells in something approaching the modern sense. It is important to realize that he homologized the cell-walls of plants with the connective tissue fibres of animals. This supposed homology seems to us so extraordinary that we do not readily understand his meaning.

Bichat (1812) devotes no less than 104 pages to the 'Système cellulaire'. What he writes at the outset (p. 11) makes it perfectly clear that by this expression he means areolar connective tissue, with the cells of which, in our modern sense, he is not at all concerned. So persistent was the term 'Zellgewebe', that Schwann himself uses it (1838*b*, col. 227) in the old sense when he wants to refer to areolar connective tissue, though in the same breath he mentions the true nucleated cells contained in it. The term 'connective tissue' is so familiar to ourselves that we may perhaps omit to reflect that it required to be invented and only gradually displaced a misleading but very familiar expression. Possibly the first use of it, in the form of 'Bindegewebe', occurs on p. 444 of Müller's *Handbuch der Physiologie* (1834), where he is discussing the histology of the kidneys, liver, &c.

The Globule-theory

What may be called the globule-theory was to some extent the forerunner of the cell-theory. Here again historians have been misled. Casual reading has suggested that various early authors knew much more about cells than in fact they did. Yet some of the 'globules' were actually cells, and to that extent the globulists were on the path of progress. The historian's difficulty is to disentangle the occasions on which they saw cells from those on which they did not.

It has already been mentioned that Swammerdam (1737–8, p. 70), in his study of the louse, stated that its viscera 'certainly consist of a mass, as it were, of globular particles (partium globulosarum), as I shall show at the proper time'. He says (p. 76) that the coats of the stomach, especially the external one, consist 'of very numerous . . . globular granules (granulis globosis)', which he describes as 'somewhat irregular'. He cannot decide whether these granules are part of the texture of the stomach or fat-particles. He remarks also (p. 70) that the muscles of the louse, when dried on glass and washed with spirits of wine, appear distinctly to be composed of globules, and also (p. 81) that the membrane that covers its nerve-ganglia seems to be composed of irregular globular particles (globosis particulis). He gives rather a confused description (pp. 84–5) of the structure of the skin, remarking that the smallest change of focus produced a new appearance. He sometimes saw 'globosae particulae' in it; sometimes it appeared to be composed of regular squares, which are illustrated in his Fig. x of Tab. II.

It is not possible to decide which of these various globules, if any, were cells and which were not. It would seem probable that he saw cells in the coats of the stomach, while the globules in the muscles may perhaps have been nuclei. Unfortunately these observations cannot be exactly dated. Boerhaave tells us in his Preface to the *Biblia* (on the page opposite p. F.2) that Swammerdam did no more scientific work after he had finished his history of the mayfly in 1675; it will be recollected that he died in 1680.

Miall (1911, p. 103), referring to the *Biblia*, says that Swammerdam 'describes a stage in which the body [of the tadpole] is entirely composed of rounded "lumps" or "granules", the *cells* of modern biology'. He repeats this on p. 106. Miall is here mistaken. On page 817 of the *Biblia* (vol. 2), Swammerdam does indeed give the impression that he knew the tadpole to be composed of cells; but it is clear from what he says at the end of the paragraph that on this occasion he is only referring to yolk-grains when he writes of 'globosas particulas'.

The year 1665 saw the first descriptions in print of what are nowadays regarded as cells of animals by Malpighi and Hooke. The former, in his *Exercitatio de omento*, refers to his microscopical examination of 'Pinguedinis globuli' and 'adiposi globuli' (reprinted in his *Opera omnia*, 1686, vol. 2, see p. 4). There would not appear to be any doubt that these were fat-cells, though it was presumably the fat-globules themselves that struck his attention, rather than the cells that contained them. Hooke (1665, p. 158) describes the hair of an Indian deer as seen under the microscope. In his figure (F in Fig. 3 of Schem. V), the imbricating scales of the cuticle of the hair are clearly seen. It looks, he says, 'like a thread of course Canvass, that has been newly unwreath'd, it being all wav'd or bended to and fro, much after that manner'. He only saw the externally projecting parts of the cuticular scales, not the complete transformed cells.

Leeuwenhoek also observed the structure of hair. He says that he examined his own hair, 'which heretofore I imagined to have seen to grow out of globuls

. . . so that Hair grows and increases by the protrusion of globuls. But two or three days agoe I observed the Hair of an *Elk*, and found it wholly to consist out of conjoyned globuls, which by my Microscope appear'd so manifestly to me, as if they could be handled' (Leewenhoeck [*sic*], 1674, pp. 23–4). Just what these globules were is uncertain; presumably they were the imbricating scales as they appeared with diffraction haloes round them.

Leeuwenhoek was now launched on his globule-finding studies, throughout which there is a curious mixture of truth and error. In the same paper he notices the 'small transparent globuls' of cow's milk, but also finds, inexplicably, that his nail consists of globules, and has no doubt that it grows from 'globuls protruded'. The most misleading of his researches in this line led to his report of the existence of globules in the brain (1686, pp. 883–9). In that of a turkey he observed 'some extream small Globules, less than 1/36th part of one of those which make the rednes in the blood'. There were also some about one-sixth of the size of a red blood corpuscle. He thought that these might have come from blood-vessels broken by himself. 'Together with the above mentioned Globules, there were some transparent irregular ones, as big or bigger than a Globule of our blood, which lay among the branches of the blood Vessels, in a space no bigger than a coarse sand.' He also found globules of various sizes in the medullary parts of the same brain, and in the brains of a sheep, ox, and sparrow.

There is nothing in this paper that could convince anyone that Leeuwenhoek saw the cells of nervous tissue. He refers more than once to his animals having been dead a considerable time before examination, and mentions (p. 885) that some of the globules seemed to consist 'of a thin transparent Oyl-like substance'. It is probable that Leeuwenhoek was looking at lipoid particles derived by maceration from cells and fibres. His description of globules in the brain, however, had a profound influence on subsequent writers.

In a letter written in 1717, Leeuwenhoek describes a transverse section of a small nerve, and gives a figure in which the fat-cells lying between the bundles of nerve-fibres are clearly seen (Leeuwenhoek, 1719, Fig. 2 on the plate opposite p. 312). He calls the fat-cells 'partes adiposae'. Monro (1726, p. 21) is probably referring to fat-cells when he describes the marrow of human bone. '. . . the Marrow,' he says, 'when hardened and viewed with a Microscope, appears like a Cluster of small Pearls. When the Oil is evaporated, these Bladders seem exactly like the *Vesiculae* of the Lungs when blown up, but not so large. The Marrow is nothing but the more oily Part of the Blood separated by the small Arteries, and deposited into these *Cellulae*.'

The foregoing observations are disconnected. With Wolff, the epigeneticist, we come to a generalization, referring to the minute structure of the embryos of animals. 'The constituent parts,' he says, 'of which all parts of the animal body are composed at their first beginnings, are globules (globuli), which always yield to a moderately good microscope' (Wolff, 1759, p. 72). He gives a figure (Fig. 1 on Tab. II) of a 28-hour chick embryo; cells can be

seen in the area pellucida. (This figure is poorly reproduced in the second edition of the book (Wolff, 1774).)

Hewson (1777, pp. 63–81) investigated the microscopical structure of various glands of the lymphatic system. 'On cutting into a fresh lymphatic gland', he says, 'we find it contains a thickish, white, milky fluid. Then if we carefully wipe, or wash this fluid from any part of it, and examine it attentively in the microscope, we observe an almost infinite number of small cells, not such as have been before described, or that have been supposed to exist in the lymphatic glands, but others too small to become visible to the naked eye, expressed Plate IV. Fig. 4.' He dilutes the fluid with a solution of Glauber's salt or with blood-serum and then sees 'NUMBERLESS small, white, solid particles, resembling in size and shape those central particles found in the vesicles of the blood'. Hewson found similar 'particles' in the thymus gland and spleen. Although both his description and his figures suggest too small a size for these particles, in relation to red blood corpuscles, yet there would not seem to be much doubt that the objects of his study were lymphocytes and thymocytes. It is curious that he calls them 'cells'. He does not suggest any relation to the cells of plants.

Leeuwenhoek's ideas on the globular structure of nervous tissue had an unfortunate influence on several workers towards the end of the eighteenth and beginning of the nineteenth century. Prochaska (1779, pp. 67–8) wrote as follows: 'For when a small piece of the substance of either the cortical or the medullary part of the cerebrum or cerebellum is placed on a very thin glass and flattened out, so that it becomes conveniently transparent, then, with the help of a selected optical lens, it is revealed to be as it were a thick paste consisting of innumerable globules (globulis), in which no movement or swimming can be observed.' Prochaska tried by dilution and prolonged maceration to separate the globules completely from one another, but without success; and he concluded that they must be held together by a very subtle and very transparent connective tissue ('tela cellulari'). He describes the shape of the globules as 'not exactly spherical but irregularly rotund' (p. 70). He figures them on Figs. VIII–XI on Plate VII.

Reference has been made by a number of historians to Prochaska's work, but it does not seem to have been carefully read by them. It is certain that Prochaska did not see nerve-cells; for his globules were less than one-eighth the size of red blood corpuscles (p. 72), and they occurred in the nerves (pp. 70, 73) as well as in the brain. Despite this, it has even been said that Prochaska saw the nuclei of nerve-cells. His Fig. X does suggest this at a glance; but he describes carefully the various appearances obtained as he focused his lens on the globules, and there can be no doubt that the figure shows merely the effect of spherical aberration. This error, together with haloes produced by lenses of small numerical aperture, must often have led the globulists astray.

Fontana (1781, p. 212) also describes 'petits corpuscules ronds' in both the cortical and medullary parts of the brain. He figures them in Fig. VI of

Plate V, but it is not possible to identify them. He differed from Prochaska, however, in his account of the structure of nerves (p. 234): here he found 'cylindres tortueux primitives', which he considered to be of an elementary nature ('des principes simples primitifs, non composés d'autres moindres'). He regarded such primitive cylinders as being the basis also of tendons and muscles. He describes globules in the 'gluten' of the skin of eels. These globules, which appear to have been cells, will be described in the discussion of Proposition II. He also found globules in the retina of the eye. (Fontana repeatedly uses the phrase 'tissu cellulaire' to mean areolar connective tissue.)

Oken must be regarded as a globulist, but there is a characteristic absence of any objective descriptions that would enable his readers to check the truth of what he says. In his remarkable work, *Die Zeugung* (1805), he develops his thesis that higher organisms are constructed from a mass of infusoria. This matter will be considered further in its proper place, in the discussion of Proposition VI. A few years later (Oken, 1809, p. 26) he makes some dogmatic general remarks on the structure of organisms:

'A sphere, of which the middle is fluid but the periphery solid, is called a *bladder* (*Blase*).

'The first organic points are little bladders (Bläschen). The organic world has for its basis an infinity of little bladders.'

Many years afterwards, when the scientific world was resounding with the fame of Schwann, Oken claimed the cell-theory for himself: 'I first instituted my doctrine that all organisms arise from and consist of *little bladders* or *cells* in my book on Reproduction (Frankfurt bei Wesche, 1805, 8vo.). These little bladders, isolated and considered in their primitive origin, are the infusorial substance or primitive slime, from which all larger organisms form themselves. . . . This doctrine of the primitive constituents of the organic substance is now generally acknowledged, and I need add nothing, therefore, to the advocacy of it' (Oken, 1843, p. iii). With a characteristic stroke of genius, Oken seems to have glimpsed the homology of the cells of plants with the globules that were coming to be recognized as frequent components of animal tissues; but his writings were not very influential, because no exact observations were recorded.

Globulism reached its zenith in the brothers Josephus and Carolus Wenzel. They were clearly influenced by Prochaska, whom they quote at length. They found 'globuli', 'cellulae', or 'corpuscula' in the brains of man, rabbit, sheep, duck, fowl, pigeon, redpoll, and carp (1812, pp. 27–36). They describe them as sub-rotund, but sometimes somewhat angular. Any tendency to suppose that they actually saw nerve-cells is opposed by their finding that nerves also are composed of similar corpuscles. They found spherical corpuscles, however, in various other tissues, and some of these may have been cells. Their general conclusions are so sweeping, and seem to forestall the cell-theory in such a surprising manner, that they deserve to be quoted; but the reader should remember the weakness of the evidence on which their generalizations are based. They conclude that:

'The cortical and also the medullary substance of the human cerebrum and also of the cerebellum;

'The substance of the colliculi that are found in the interior of the human cerebrum;

'The substance of the pineal gland, of the spinal medulla, and of the nerves;

'Finally, the mass of the cerebrum in mammals, birds and fishes, consist of the same small, mutually coherent, sub-rotund corpuscles, of which the substance of muscle, liver, spleen, and kidneys is composed.'

Their final conclusion is that 'the particular structure of the whole of the cerebrum and nerves and also of all the other organs is cellular (cellulosam).... Finally, that the principle (Principium), or fundamental structure, of all the solid parts without exception is one and the same.'

Meckel (1815) was the first to incorporate the globule-theory in a text-book of anatomy. He regarded organisms as made up of two ultimate constituents: globules (Kügelchen), and 'a *coagulated*, or *coagulable* and therefore *plastic* substance' (1815, p. 4), in which the globules are invariably embedded. The globules are not always exactly spherical, but they are never angular. Apart from the blood, in which they assume particular shapes, all the globules of the body of any one species of animal have the same form; they are never elongated in one part and round in another. In man they are round. They are larger in the spleen than in the kidneys, and in the latter than in the liver. The milk globules are of the same nature as those of other organs.

Treviranus's contribution (1816) to the globular theory was not important. He remarks that all good observers see the globules (Kügelchen) in the brain. He recognizes three main elements in the tissues of animals (p. 140): elementary cylinders, protein globules (Eyweisskügelchen), and formless material. He also recognizes elementary fibres, which appear to have been connective tissue fibres, and which he regarded as of plant-like nature; but he denies (p. 126) that there is any trace in animals of anything resembling the cellular tissue of plants. It is difficult to identify Treviranus's Kügelchen; in some cases they may have been nuclei, in others artifacts, in *Hydra* nematocysts. His figures are unhelpful and show nothing that can be recognized as a cell.

If Treviranus's contribution is confused, Home's (1818, 1821) is simply erroneous. His studies were made in collaboration with a Mr. Bauer, who seems to have done most of the practical work. They noticed (1818) that when blood coagulates, the red blood corpuscles tend to unite in lines. They then boiled or roasted voluntary and involuntary muscle, macerated it in water, and found that the fibres 'are readily broken down into a mass of globules of the size of those in the blood, deprived of their colour' (1818, p. 175). They concluded that muscle is formed by the joining together of red blood corpuscles in lines, and suggested that nerve-fibres were formed in the same way. In a later paper (1821) Home describes both nerves and brain as containing innumerable globules, from 1/2,000 inch (6μ) to 1/4,000 inch in diameter. He evidently considered them to be derived from the red corpuscles of the blood. It is strange that Prevost and Dumas (1821), who

were later to make an important advance in science by the discovery of cleavage, agreed with Home's opinion that muscle fibres are formed by the arrangement of red blood corpuscles in lines.

Heusinger (1822) shows affinity with Home, and indeed carries his ideas farther. He considered (pp. 113–16) that the tissues of the body had three constituents: formless matter, globules (Kugeln), and bladders (Blasen). The first he regarded 'as the mother, as the primitive sea of all other tissues'. Fibres are formed by the arrangement of globules in a row: bladders arise from globules by the development of a differentiated pellicle, and themselves give rise to vessels by arrangement in rows and confluence of their cavities. Heusinger generalizes freely and gives little precise information: his style is reminiscent of Naturphilosophie.

Milne Edwards (1823) was strongly influenced by Prochaska, Fontana, and Wenzel. He made a systematic study of the microscopical structure of many organs. He found 'globules' in the connective tissue of man and various animals, in the peritoneum, conjunctiva, the mucous membrane of the intestine, voluntary muscle, tendon, skin, the walls of arteries and veins, and in the white and grey matter of the brain. In nearly every case he notes that the globules are 1/300 mm. in diameter. Unfortunately he gives no figures, and it is impossible to guess exactly what he saw. In some cases he may have been looking at nuclei, in others at lipoidal droplets, in others again he may have seen cells; but if he did, it is difficult to account for their uniformly spherical shape and minute and unvarying size.

The last in the direct succession of the globulists was Dutrochet (1824). Himself mainly a botanist, he relied to a large extent on Milne Edwards for his information about the microscopical structure of animals; but he claims (p. 201) to have verified the latter's observations. For him, 'all the organs of animals are composed of agglomerated globular corpuscles' (p. 13; see also pp. 200–1).

Although there was some truth in the claims of the globulists, and although they did pave the way for a true understanding of the microscopical structure of animals, yet some check to their errors was urgently needed. It was provided by Hodgkin and Lister (1827). Using the improved microscope designed by Lister, they found no globules, but only fibres, in striated muscle and in the muscle of arteries. They looked in vain for globules in nerves. They saw no globules in brain, but only very small particles, which they regarded as resulting from the disintegration of the tissue. They saw no globules in connective tissue (or 'cellular membrane', as they called it). They found human red blood corpuscles to be concave, and the particles of pus to be irregular in shape. They found globules only in milk. They were aware that their results differed from those of Milne Edwards, who was a friend of Hodgkin, and attributed the difference to the imperfection of Edwards's microscope. There can, indeed, be little doubt that many of the globules reported by the early microscopists were images of minute particles, smaller than any ordinary cells, but surrounded by haloes. The fact that the

excesses of the globulists were exposed by Lister's microscope seems significant; for the particular advantage of his instrument was that spherical aberration was corrected and the 'ring' appearance round small particles thus reduced. His objectives, though not perfected by this time, must already have been good. The work of Hodgkin and Lister was a healthy and much-needed corrective. They were supported by Grainger (1829), whose own observations agreed with theirs.

The time had now arrived when microscopists were beginning to see actual cells in various animal tissues. Von Baer (1828, pp. 144–5) noticed that the elements of which an embryo consists—fibres, globules, and platelets—become smaller as development proceeds. He uses the words Kügelchen and Körnchen interchangeably. He says of the Körnchen in the developing chick that they 'are so large, in relation to the parts that they compose, that one might say that the embryo at a very early stage resembles a picture made of paving-stones or blocks of granite. On the first day the notochord consists almost entirely of one row of such globules (Kügelchen), which one can count with tolerable accuracy . . . the individual globules can be distinguished in the embryonic area with moderate magnification; the embryo appears to contain several hundreds of them.' There is no reason to doubt that these Körnchen and Kügelchen were cells.

Dutrochet (1837) illustrated a small fragment of the brain of the frog as seen under the microscope. The figure (Fig. 3 on Plate 30) shows a large number of cells and a small vessel running among them. They were probably nerve-cells, though the figure does not permit this to be concluded with certainty. It must be allowed that Dutrochet was not an exponent of animal histology. He remarks (p. 470) that 'when observing with the microscope the tissue of the brain, the liver, the kidneys, the spleen, etc., in a frog, for instance, one really notices no difference'. Purkinje (1838) found that the most diverse organs consist of Körner, Körnchen, or Cylinderchen, often associated with fibres. He found this to be true of the glands of the mammalian stomach, the liver, the salivary glands, the pancreas, unspecified mucous glands, the ear-wax glands, kidneys, testes, epididymides, epidermis, mucous epithelia, ciliated membranes of the respiratory tract and of the female genital system, spleen, thymus, thyroid, and lymph-glands. His paper has a more modern aspect than those of the globulists, and there is reason to believe that he saw the cells of most or all of these organs. The Cylinderchen were the cells of columnar epithelium. By this time, however, the nucleus was beginning to be identified in various tissues. This provided a criterion by which a cell could be recognized as such. The subject now falls within the scope of Proposition II, and will be discussed further under that heading in the second part of this series of papers.

Early Comparisons of Plant and Animal Cells

When considering plant tissues, most of the early observers concentrated their attention upon the cell-walls, which they thought to form a continuous

meshwork. Either this meshwork corresponded to the fibres of connective tissue, as Lamarck and the others supposed, or else it seemed to have no counterpart at all in the animal kingdom, in which the tissues consisted largely of 'globules'. Microscopists were slow to realize that the utricles held in the meshes of plant cell-walls might correspond to the globules.

The purpose of this section of the paper is to give some early examples of cases in which actual cells of plants and animals were compared.

Oken's generalizations on this subject have already been quoted (p. 118). It is impossible to be sure that he saw the cells of animals.

Dutrochet (1824, pp. 14–15) said that there is a 'similitude évidente' between the microscopical structure of the brain of gastropods on the one hand and of the pith of *Mimosa pudica* on the other. Raspail (1833, pp. 187, 191) compared the microscopical structure of fat with that of plant tissue. He speaks of 'the analogy of this animal cellular tissue with vegetable cellular tissue'. Valentin (1835, p. 287) described the mesoderm (Gefässblatt) of the chick embryo as composed of large Kugeln, so tightly crowded together 'that they are flattened at many points of contact and often, as [in] the cellular tissue of plants, assume a hexagonal form'. Valentin (pp. 209–10) also refers to a condition resembling the cellular tissue of plants in the ossifying cartilage of the labyrinth of the ear, but it is doubtful whether he is here referring to cells. Müller (1835, p. 25) wrote as follows of the notochord of *Myxine glutinosa*, as seen in transverse section under the microscope: 'The cells are irregular, and unlike one another, but resemble the cells of plants to some extent in that the walls seem to be closed on all sides and mostly touch one another in straight lines, so that irregularly polygonal figures appear in transverse sections.' Müller shows this in Fig. 1 of Plate IX.

Turpin (1837) was led to compare the cells of plants and animals when he undertook a critique of the microscopical studies of a certain Dr. Donné on the liquids secreted and excreted by organic tissues. To check the accuracy of Donné's statements, Turpin repeated most of the observations. Donné had described what were evidently squamous epithelial cells of the human vagina. Turpin says (p. 210): 'After having thoroughly studied the vesicles forming the layer of mucus produced by the vaginal mucous membrane, one cannot avoid seeing in it a cellular tissue that is well-organized and composed, like all vegetable cellular tissues, of an agglomeration by simple contiguity of distinct vesicles living *individually* each on its own account at the expense of the mucous fluid that bathes them on all sides. This animal cellular tissue . . . may be rigorously compared with that of many vegetable cellular tissues.'

Dutrochet reverted to the comparison of plant and animal cells many years after making his first contribution to this subject. He made a direct comparison between the cells of plants and those of the salivary gland of *Helix* (1837, pp. 469–70). 'One sees from that', he remarks, 'that nature possesses a uniform plan for the intimate structure of organised beings, both animal and vegetable.'

As a result of the extensive histological researches that have already been mentioned, Purkinje (1838, p. 175) also drew a comparison of plant and animal cells. 'Consequently,' he wrote, 'the animal organism almost completely reduces itself to three main elementary forms: the fluid, the granular (körnige), and the fibrous. The granular ground-form suggests again an analogy with the plant, which, as is well known, is almost entirely composed of granules or cells (Körnern oder Zellen).'

Comment

The following is the essence of the ideas briefly summarized in the first proposition:

The tissues of most organisms, when examined under the microscope, are seen not to be perfectly continuous, for there is generally a partitioning by cell-membranes, cell-walls, and intercellular matter of various kinds; and this partitioning leaves much of the material of the organism in the form of more or less separate bodies to which the name *cells* is applied. These cells are usually of relatively simple shape in the less differentiated tissues (spheroids, simple polyhedra, &c.).

The facts recorded in this first paper are mainly of historical interest, for the truth of the first proposition is generally admitted and little in the way of critique is possible. It must be remarked, however, that although the knowledge summarized in the proposition was fundamental for the establishment of the cell-theory, yet those who got the knowledge were not at the time in a position to envisage the large superstructure that would eventually be built on their foundations. The first necessary advance was the production of evidence that the various objects that were called cells had in fact important characters in common that made it proper to include them all under a single name. That is the subject of the second proposition which will be considered in the second of this series of papers.

REFERENCES

Awerinzew, S., 1910. Biol. Centralbl., **30**, 465.

v. Baer, K. E., 1828. *Über Entwickelungsgeschichte der Thiere. Beobachtung und Reflexion.* Erster Theil. Königsberg (Bornträger).

Baitsell, G. A., 1940. Amer. Nat., **74**, 5.

Bichat, X., 1812. *Anatomie générale, appliquée à la physiologie et à la médecine.* New edit. Vol. 1. Paris (Brosson, also Gabon).

Bourne, G. C., 1895. Quart. J. micr. Sci., **38**, 137.

—— 1896*a*. Sci. Prog., **5**, 94.

—— 1896*b*. Ibid., 227.

—— 1896*c*. Ibid., 304.

Burnett, W. J., 1853. Trans. Amer. Med. Assoc., **6**, 645.

Conklin, E. G., 1939. Amer. Nat., **73**, 538.

Dobell, C. C., 1911. Arch. Protistenk., **23**, 269.

Dutrochet, M. H., 1824. *Recherches anatomiques et physiologiques sur la structure intime des animaux et des végétaux, et sur leur motilité.* Paris (Baillière).

—— 1837. *Mémoires pour servir à l'histoire anatomique et physiologique des végétaux et des animaux.* 2 vols. and atlas. Paris (Baillière).

E(VELYN), J., 1664. *Sylva, or a Discourse on Forest-trees, and the Propagation of Timber in His Majesties Dominions.* London (Martyn and Allestry).
FONTANA, F., 1781. *Sur les poisons et sur le corps animal.* 2 vols. Paris (Nyon); London (Emsley).
FOSTER, M., 1901. *Lectures on the History of Physiology during the Sixteenth, Seventeenth and Eighteenth Centuries.* Cambridge (University Press).
FRÉDÉRICQ, L., 1884. *Théodore Schwann: sa vie et ses travaux.* Liége (Desoer).
FULINSKI, B., 1916. Zool. Anz., **47,** 381.
GEROULD, J. H., 1922. Sci. Mon., **14,** 268.
GREW, N., 1672. *The Anatomy of Vegetables begun. With a General Account of Vegetation founded thereon.* London (Hickman).
—— 1682. *The Anatomy of Plants. With an Idea of a Philosophical History of Plants. And Several Other Lectures, read before the Royal Society.* Publ. by the author (place not stated).
HALLER, A., 1757. *Elementa physiologiæ corporis humani.* Lausannæ (Bousquet).
—— 1779. *First Lines of Physiology.* Transl. from the correct Latin edition. Edinburgh (Elliot).
HERTWIG, O., 1893. *Die Zelle und die Gewebe. Grundzüge der allgemeinen Anatomie und Physiologie.* Jena (Fischer).
HEUSINGER, C. F., 1822. *System der Histologie.* Eisenach (Bärecke).
HEWSON, W., 1777. *Experimental Enquiries: Part the Third.* Edit. by M. Falconar. London (Longman).
HODGKIN, —, and LISTER, J. J., 1827. Phil. Mag., **2,** 130.
HOME, E., 1818. Phil. Trans., **108,** 172.
—— 1821. Phil. Trans., **111,** 25.
HOOKE, R., 1665. *Micrographia: or Some Physiological Descriptions of Minute Bodies made by Magnifying Glasses. With Observations and Inquiries thereupon.* London (Martyn & Allestry).
—— 1678. *Lectures and Collections made by Robert Hooke, Secretary of the Royal Society.* London (Martyn).
KARLING, J. S., 1939. Amer. Nat., **73,** 517.
LAMARCK, J.-B.-P.-A., 1809. *Philosophie zoologique, ou exposition des considérations relatives à l'histoire naturelle des animaux.* Paris (Dentu, and from the author).
LEEWENHOECK [*sic*], 1674. Phil. Trans., **9,** 23.
LEEUWENHOEK, A., 1686. Phil. Trans., **15,** 883.
—— 1719. *Epistolæ physiologicæ super compluribus naturæ arcanis.* (Vol. 2 of the *Opera omnia.*) Delphis (Beman).
—— 1722. *Opera omnia.* Vol. 1 of Editio novissima. Lugduni Batavorum (Langerak).
MALPIGHI, M., 1675. *Anatome plantarum. Cui subjungitur appendix, iteratas & auctas ejusdem authoris de ovo incubato observationes continens.* London (Royal Society).
—— 1686. *Opera omnia, figuris elegantissimis in æs incisis illustrata.* 2 vols. Londini (Sawbridge).
MARK, E. L., 1879–80. Bull. Mus. Comp. Zoöl. Harvard, **6,** 173.
MECKEL, J. F., 1815. *Handbuch der menschlichen Anatomie.* Erster Band. Halle and Berlin (Buchhandlungen des Hallischen Waisenhauses).
MIALL, L. C., 1911. *History of Biology.* London (Watts).
—— 1912. *The Early Naturalists: Their Lives and Work (1530–1789).* London (Macmillan).
MILNE EDWARDS, H., 1823. *Mémoire sur la structure élémentaire des principaux tissus organiques des animaux.* Paris (Migneret).
M'KENDRICK, J. G., 1888. Proc. Phil. Soc. Glasgow, **19,** 71.
MOLDENHAWER, J. J. P., 1812. *Beyträge zur Anatomie der Pflanzen.* Kiel (Wäser).
MONRO, A., 1726. *The Anatomy of the Humane Bones.* Edinburgh (Ruddiman).
MÜLLER, J., 1834. *Handbuch der Physiologie des Menschen für Vorlesungen.* Erster Band. Coblenz (Hölscher).
—— 1835. *Vergleichende Anatomie der Myxinoiden, der Cyclostomen mit durchbohrtem Gaumen.* Erster Theil. Berlin (Druckerei der Königlichen Akademie der Wissenschaften).
OKEN, (L.), 1805. *Die Zeugung.* Bamberg and Wirzburg (Goebhardt).
—— 1809. *Lehrbuch der Naturphilosophie.* Erster und zweiter Theil. Jena (Frommann).
—— 1843. Ibid. 3rd edit. Zürich (Schulthesz).
POLLENDER, A., 1868. *Wem gebührt die Priorität in der Anatomie der Pflanzen dem Grew oder dem Malpighi?* Bonn (Georgi).

PROCHASKA, G., 1779. *De structura nervorum. Tractatus anatomicus tabulis aeneis illustratus.* Vindobonae (Graeffer).
PURKINJE, —, 1838. Ber. Versamm. deut. Naturf. Aerzte, **15**, 174.
RÁDL, E., 1930. *The History of Biological Theories.* Oxford (University Press).
RASPAIL, F. V., 1833. *Nouveau système de chimie organique, fondé sur des méthodes nouvelles d'observation.* Paris (Baillière).
REMAK, R., 1855. *Untersuchungen über die Entwickelung der Wirbelthiere.* Berlin (Reimer).
RIES, E., 1943. Fort. Zool., **7**, 1.
SACHS, J. v., 1890. *History of Botany* (1530–1860). Transl. by H. E. F. Garnsey. Oxford (Clarendon Press).
SCHLEIDEN, J. M. [*sic*], 1849. *Principles of scientific botany: or, botany as an inductive science.* Transl. by E. Lankester. London (Longman, Brown, Green & Longmans).
—— M. J., 1838. Arch. Anat. Physiol. wiss. Med. (no vol. number), 137.
SCHWANN, T., 1838*a*. Neue Not. Geb. Nat. Heilk. (Froriep), **5**, col. 33.
—— 1838*b*. Ibid. 225.
—— 1839*a*. *Mikroskopische Untersuchungen über die Uebereinstimmung in der Struktur und dem Wachstum der Thiere und Pflanzen.* Berlin (Sander'schen Buchhandlung).
—— 1839*b*. (Contribution to Wagner, 1839.)
—— 1847. *Microscopical Researches into the Accordance in the Structure and Growth of Animals and Plants.* Transl. by H. Smith. London (Sydenham Society).
—— 1884. (Original words of Schwann, not known to be printed elsewhere, given by Frédéricq (1884).)
SEDGWICK, A., 1894. Quart. J. micr. Sci., **37**, 87.
—— 1895. Ibid., **38**, 331.
STIRLING, W., 1902. *Some Apostles of Physiology.* London (Waterlow).
STRASBURGER, E., 1880. *Zellbildung und Zelltheilung.* 3rd edit. Jena (Fischer).
SWAMMERDAM, J., 1737–8. *Biblia naturae; sive historia insectorum, in classes certas redacta.* With a preface by H. Boerhaave. Leydae (Severinum, Vander, Vander).
—— 1758. *The Book of Nature; or, the History of Insects: Reduced to Distinct Classes.* Transl. from the Dutch and Latin original edition by T. Flloyd. London (Seyffert).
TREVIRANUS, G. R. and L. C., 1816. *Vermischte Schriften: anatomischen und physiologischen Inhalts.* 1 Band. Göttingen (Röwer).
TURNER, W., 1890*a*. Nature, **43**, 10.
—— 1890*b*. Ibid., 31.
TURPIN, —, 1837. Ann. sci. nat., **7**, 207.
TYSON, J., 1870. *The Cell Doctrine: Its History and Present State.* Philadelphia (Lindsay & Blakiston).
—— 1878. Ibid. 2nd edit. Philadelphia (Lindsay & Blakiston).
VALENTIN, G., 1835. *Handbuch der Entwickelungsgeschichte des Menschen mit vergleichender Rücksicht der Entwickelung der Säugethiere und Vögel.* Berlin (Rücker).
VIRCHOW, R., 1859. *Die Cellularpathologie in ihrer Begründung auf physiologische und pathologische Gewebelehre.* Berlin (Hirschwald).
WAGNER, R., 1839. *Lehrbuch der Physiologie für akademische Vorlesungen und mit besonderer Rücksicht auf das Bedürfniss der Aerzte.* Leipsig (Voss).
WALDEYER, W., 1888. Arch. mikr. Anat., **32**, 1.
WEISS, P., 1940. Amer. Nat., **74**, 34.
WENZEL, J. and C., 1812. *De penitiori structura cerebri hominis et brutorum.* Tubingae (Cottam).
WHITMAN, C. O., 1893. J. Morph., **8**, 639.
WILSON, J. W., 1944. Isis, **35**, 168.
WOLFF, C. F., 1759. *Theoria generationis.* Halae ad Salam (Hendel).
—— 1774. Ibid. New edit. Halae ad Salam (Hendel).
WOODRUFF, L. L., 1939. Amer. Nat., **73**, 485.

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